Revamping of Cotton Breeding Programs for Efficient Use of Genetic Resources under Changing Climate

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Abstract: Empirical cotton breeding was based upon the concept of selecting single and best high-yielding progeny from the segregating populations to develop a cultivar. It helped in releasing of high yielding varieties with superior fibre quality through conventional breeding. Though the production of cotton has been increased manifold in the last decades, but it has also seen enormous ebb and flow of yield uncertainties during the past several years. Therefore, the development of climatic resilient cotton ideotypes through the introduction of wild alleles and using contemporary genetic markers have become inevitable. Emerging genome engineering technologies such as CRISPR/Cas9 system can also potentially exploited to edit the disease susceptible and negative regulators of yield related genes in cotton. In the present review we concentrate on accomplishments and forthcoming of plant breeding and biotechnology to facelift the cotton breeding programs.

Keywords: genome editing; introgression; lint yield; plant breeding; wild germplasm

1. Introduction

Cotton is a resourceful crop being grown to provide raw material to the textile industry. It is cultivated on approximately 3% of the total world arable land (ICAC https://icac.org/). The leading cotton producing countries are India, China, USA, Brazil, and Pakistan (Figure 1). To date, the improvement in cotton crop have been achieved through releasing improved cultivars with better yield potential, higher fibre qualities and capacity to tolerate changing climatic conditions along with early maturity, high fertilizers and water use efficiency [1]. Most of these varieties were developed by selecting superior plants from narrow germplasm. Moreover, the efficiency of selection also depends upon numerous factors, i.e., heritability, genetic advance, and the environment [2]. Since most of the economically important traits are polygenic in nature and is being highly influenced by the environment. Therefore, marker assisted breeding and whole-genome sequencing technologies have led to the identification of genes and quantitative trait loci (QTLs) linked with traits of interest [3]. Genetically modified (GM) cotton is considered to be one of the earlier transgenic crops commercially released during 1996 [4]. Transgenic cotton has provided the resistance against bollworm complex and minimize the use of pesticides and herbicides [5]. The success of transgenic cotton diverted the attention of seed and biotechnological enterprises for the development of improved GM cotton with

novel resistance genes [6]. Research and field assessment of abiotic stress-tolerant transgenic cotton is also under process and anticipating commercializing in a coming years. Functional genomic databases facilitate the mining of valuable genes and markers linked to those for the identification of important metabolic pathways involved in lint quality, oil and protein contents [7]. Here we discussed the breeding strategies, genomic selection and biotechnological approaches to improve cotton breeding programs. We advocated for revamping of cotton breeding programs in tandem with whole genome sequencing databases and gene editing technologies to develop next-generation genetically modified cotton cultivars, collectively setting a stage for sustainable production under yield uncertainties, ever-increasing effects of climate change and socio-economic needs (Figure 2).
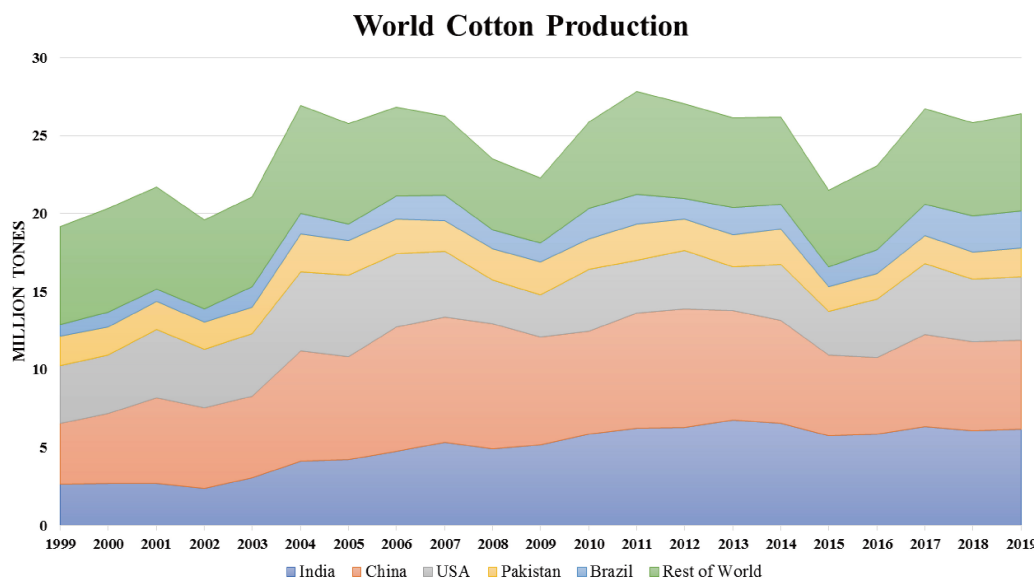
Figure 1. World cotton production status in last two decades. The data is retrieved from OECD/FAO (2019), "OECD-FAO Agricultural Outlook", OECD Agriculture statistics database.
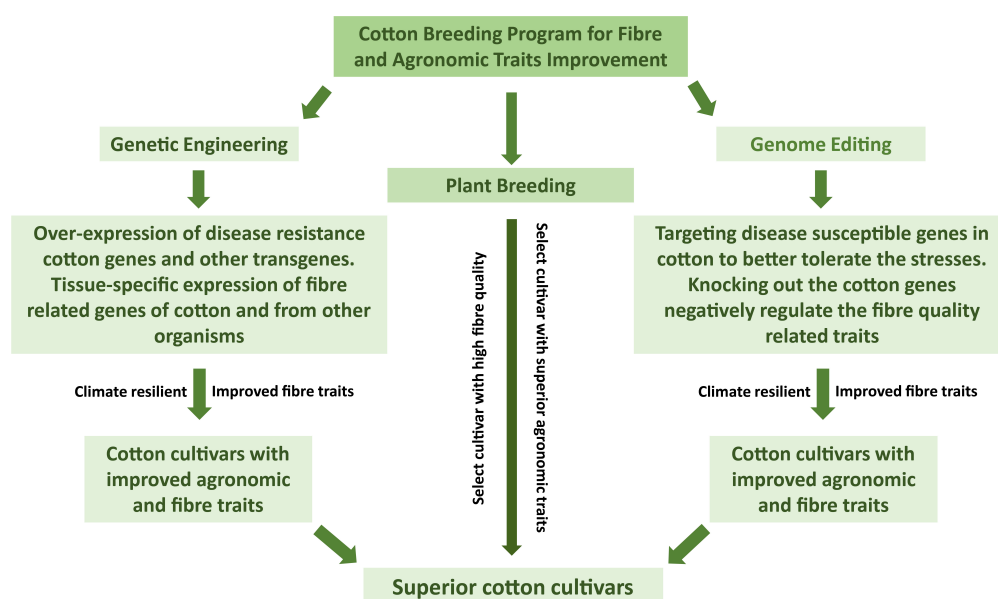
Figure 2. Proposed revamped cotton breeding program. Fine-tune the already present traits and add new traits to cultivated cotton varieties through plant breeding, genetic engineering, and genome editing tools. Pyramiding of useful traits by crossing between genetically altered and elite cultivars to develop climate resilient cotton cultivars.

2. Concept of Sustainable Cotton Production

Cotton fibre yield is the outcome of collected bolls, their weight and lint percentage from a certain plant population over a single (determinate type) or multiple (indeterminate type) picking events depends upon growth habit of cultivar [8] However, the yield is also dependent upon various other traits and sustainable production can only be achieved by modifications and improvement in such attributes. Production can be sustained by preserving biodiversity, minimizing the discharge of soil nutrients along with improving adaptability, quality, input use efficiencies and resilience to environmental calamities [9]. Moreover, precision agriculture technologies are now also being implemented for the optimum use of farm resources [10]. If a new technology works out to improve crops without affecting the environment, then it is appropriate to adapt to enhance the farming profit. Therefore, a combination of empirical breeding, biotechnological tools along with various better agronomic practices and adaptation to mechanized technologies is requisite for the sustainable farming of cotton.

3. Cotton Genetic Resources

The genus *Gossypium* is comprised of more than 50 species, out of which 46 species are wild in nature while four are cultivated species [11]. The narrow genetic base of cultivated cottons is the evidence of bottleneck events in the course of evolution, domestication of land-races, and intensive breeding via artificial selections [12]. The cultivated allotetraploid cotton (*G. hirsutum*) is evolved from two divergent diploid species where the donor of A genome is still under investigation while D genome is contributed from *G. raimondii* [13]. Cotton have eight highly divergent genomes, i.e., A genome is native to Africa and Asia, B genome from Africa and Cape Verde Islands, C and D genome evolved in Australia and Mexico, respectively. E genome is native to Arabian Penisula, Northeast Africa, Southwest Asia and F genome evolved in East Africa. G genome is native to Australia while the K genome is native to NW Australia and Cobourg Peninsula [14]. Limited information is available about the nature of regulatory evolution which follows crop domestication for polyploid crops such as cotton. Regulatory variation is strongly correlated with genome evolution, patterns of parental expression inheritance, properties of the co-expression gene network, and the genomic loci responsible for domesticated traits [15]. Although *G. hirsutum* was grown continuously after the polyploidization event and is better suited to grating environmental conditions and produce a more lint yield than G. barbadense [16]. But the domestication of upland cotton also leads to several genetic consequences including genetic erosion [17].

Several phenotypic and molecular data have also confirmed the occurrence of low genetic diversity in cotton germplasm [18,19]. But comprehensive evidence for low molecular genetic diversity of cotton is still under investigation [16]. So, there is a dire need to broaden the genetic base of existing cotton germplasm through wide hybridization and mutation [20]. The knowledge of geographic distribution and origin of genomes can potentially be exploited to broaden the distinctiveness and adaptability of cultivated cotton [21,22]. The wild cotton germplasm provide novel genes related to stress tolerance, adaptability, fibre quality, oil content, and cytoplasmic male sterility to induce in cultivated lines [23].

In past, synthetic cultivars were rebuilt from *G. raimondii* and *G. herbaceum* species, but linkage-drag was the major drawback to transfer valuable genes from those experimental lines into the cultivars. Due to recombination, linkage's disequilibrium breaks up creates different allelic combinations, affects natural crop selection, and plays a significant part in crop domestication and improvement [24]. Linkage-drag reduced the yield, lint quality, disturbed several gene regulatory systems, created chromosomal abnormalities, hybrid run-out and genome assortments [25]. To break such linkage-drag, a set of chromosomal segment introgression lines (CSILs) has been developed by interspecific hybridization between Egyptian and American cotton [26]. Several other CSILs are also under development. These CSILs can be used for fine QTLs mapping [27].

Due to the limited genetic diversity in cotton compared to many other crop plants, and the associated genetic complications, the latest state of the art genomics technologies significantly assist fundamental and advanced cotton research [16]. High-quality genome assembly of *Gossypium* species is

a daunting challenge because of their large and complex genomes. Exploring the genetic and molecular basis of the origin of *Gossypium* species, diversification, speciation, and advancing genomic-assisted breeding requires thorough analysis and a deep understanding of the genome organization [12]. The lack of accurate genomic data has been a significant drawback for developing improved cotton cultivars.

Advancement in sequencing technologies has strengthened the field of genomics and supported the dissection and manipulation of useful agronomic traits. It also demonstrated the genomic evolution of polyploidy crops [28]. The presence of a large amount of cotton sequence data set flickered the scientist to have an integrated cotton functional genomics resource. Currently existing large set of cotton databases includes cotton database resource developed by International Cotton Genome Initiative (ICGI) (https://www.cottongen.org/), Cotton Functional Genomics Database (https://cottonfgd.org/) [7]. and the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/genome?term=Gossypium+hirsutum%5Borgn%5D&cmd=DetailsSearch). These databases facilitate simple, rapid, and convenient access to the required information related to *Gossypium* species. Thus, the genomic information facilitates the breeding by design for robust approaches including fine QTL mapping to find the allelic variation of mapped loci and use of selected loci in cotton breeding programs [29]. Recent advances in functional genomics methods led the development of fast trait mapping and cloning methods like BSR-Seq [30], genome-wide association [31], MutMap [32], QTLseq [33], Indel-seq [34], and map-based cloning [35]. Therefore, a combination of these methods along with multi-omics and reverse genetic approaches accomplish the identification and cloning of agronomically important genes [12]. One such example is the identification of *GhHOX3* transcription factor, which regulates the elongation of cotton fibres. Overexpression of *GhHOX3* leads to longer fibre, while its silencing reduces the fibre length by more than 80% [36].

The mapping of RNA sequencing data set is also an additional remarkable application of cotton genomics to identify the differentially expressed genes under various circumstances [37]. Furthermore, the accessibility of reference cotton genome sequences provide insights into the fiber quality attributes such as length and fineness. Although several innovations are reported due to the use of genome assembly methods but haplotypes are not developed so far due to heterozygosity, large genome size and repetitive sequences in the cotton genome. To overcome these impediments, use of PacBio and Oxford Nanopore technologies is a way forward.

4. Classical Cotton Breeding Concept

Before the era of genomics and molecular markers, plant breeding was a "number game". The greater number of crosses, the more chances to discover superior recombinants. This fundamental strategy was based upon the idea of "cross best with the best likewise select the best and expect the best" [38]. Such breeding approaches were efficiently used for the development of cultivars with higher yield and superior fibre quality attributes in cotton [39]. In the contrary, those breeding plans also gave rise to homogeneity and drained genetic diversity from the germplasm. Production of hybrid cotton seed for commercial cultivation lifted the yield barriers to a greater extent but attained limited attention among seed companies due to the high cost of production and poor seed setting in interspecific crosses [40]. Making large number of random mutant populations either through physical or chemical mutagens before the selection of desirable plants had played a remarkable role in improving cotton yield [41]. Some impressive achievements were reflected after the release of NIAB-78, Lumian No.1, and MA-9 cotton cultivars for general cultivation in Pakistan, China and India, respectively [42]. However, mutation breeding still had limited scope due to less frequency of desirable alleles, large population screening and pleiotropic effects. Although the introduction diversity by various means like site-specific, mutation, and wide hybridization is requisite [43,44]. The breeding challenges are also emerging than before, the use of modern day biotechnological and genomics tools has become indispensable. So, it is imperative to use cotton genome sequence information and deploy biotechnological tools in breeding programs to further augment the lint yield and quality in a short time frame.

5. Tolerance to Environmental Stresses

Global cotton production has been intimidated by various biotic and abiotic stresses among that insects/pest infestation, bacterial/viral/fungal diseases, heat, salinity, and drought stresses are the major impediments in high lint yield. Biotic stresses in cotton caused up to 30% losses worldwide annually [45]. Among various biotic stresses in cotton, whitefly transmitted cotton leaf curl disease (CLCuD) is considered to be one of the major diseases of cotton crop due to its rapid spread and virus genome plasticity. Afterward, the infestation of cotton bollworms is, found to be a major pest of cotton crop and now has been aptly controlled by the introduction of Bt cotton [46]. In addition, some new problems have arisen as a result of the ongoing cultivation of Bt cotton that leads to the development of insects/pest resistance to Bt toxins [47]. For instance, pink bollworm resistance breakdown has been spotted against Bt cotton in India [48]. Thus, to counter the insect resistance and disease management, different strategies were also utilized include; proteinase inhibitors, engineering of other toxins, proteases, and double-stranded RNA technology [49–52]. Protease inhibitors (PIs) have been identified as a key plant defense player against insect herbivores. Transgenic tobacco overexpressing the tomato and potato receptor II PIs greatly delayed insect growth compared with non-transgenic plants [53]. Many other toxins with insecticidal activity may contribute to the arsenal of engineered Bt toxins. For example, an α-amylase inhibitor gives resistance to pea weevil in Pisum sativum and mannose-binding lectins, especially *Galanthusnivalis agglutinin* (GNA), are toxic to various insects in monocot plants [54,55]. Studies have discovered considerable strides over the last three decades in discovering and unraveling many facets of the dsRNA mediated gene silencing. The engineered dsRNA as an active ingredient for formulations of biopesticides is considered a raw material which can be applied to different purposes such as the pest management. A proof of concept research revealed that a dsRNA construct in a genetically manipulated maize plant may induce larval mortality in *Diabrotica virgifera*, the western corn rootworm (WCR) [56].

Among abiotic stresses, drought and heat are the major threats to cotton growth and development. The rise in the CO_2 level has changed the annual day and night temperature. It can reduce the plant's ability to retain the bolls and caused 40% cotton bolls abscission [57]. Additionally, high temperature also had adverse effect on viability of pollen, antioxidant activities, plant physiology, plant morphology, yield, and quality of fibre [58,59]. Heat stress also boosted the evapotranspiration losses which increase the crop water requirement. Several candidate genes were previously mined within the QTL region related to chlorophyll content, cell membrane stability, and saturated leaf weight. Numerous miRNAs, such as miR164, are associated with NAC and MYB genes, which have important role in improving drought tolerance in cotton [60]. Such findings provide an opportunity to use these QTLs/genes in breeding programs to developing climate-resilient cotton cultivars.

Screening of domesticated and wild cotton germplasm for various agronomic traits drive the cotton breeding programs by hybridizing these attributes. Also, fine QTL mapping of these attributes associated with heat and drought stress tolerance can speed-up the hybridization process and decrease the linkage-drags in the advanced breeding lines [60,61]. As there were many other agronomically important genes found using forward and reverse genetic approaches. The integration of these genes has brought no yield gaps because they are not negatively associated with yield related traits. Previously, the breeders had made considerable efforts to develop the heat and drought-tolerant cotton cultivars. The efforts of the cotton breeders were usually meant to increase the yield of cotton under the growing climatic changes. Table 1 offers a few possible ideotypes for cotton plants under various challenging conditions.

Table 1. Ideotypes of cotton for various breeding objectives.

Breeding Trait	Indeotypes	References
Heat tolerance	Efficient canopy architecture, capable to hold flower and retain bolls at lower nodes, leaf angle and thickness.	[62]
Drought tolerance	Early maturity, deep root system, small leaf area, improved stem reserve mobilization, high cuticular waxes.	[63]
Insect resistant	Small and okra type, and hairy leaves, long frego bract, red colored leaves and stem, extra glands in leaves.	[64]
Ultra-low gossypol in seed	Ultra-low gossypol contents in seed, tissue specific suppression of genes involved in gossypol synthesis in seed.	[65]
Early maturity	Swift vegetative growth phase, efficient and quick flowering, plant height to node ratio, boll retention at first fruiting branch.	[66]
High yield potential	Compact canopy type, extended reproductive growth, high photosynthetic rate, increase number of bolls per plant, effective boll opening, earlier anthesis.	[67]
Mechanized farming	Small to medium compact canopy type, early reproductive growth, early maturing, uniform flowering and fluffy boll opening.	[68]

6. New Emerging Technologies

Transgenic crops are now being grown over an area of 191.7 million hectares after more than two decades of commercialization [69]. The majority of the genes introduced to develop transgenic crops were related to herbicide and insect resistance. Globally, transgenic cotton is cultivated on about 70% (189.9 M ha) of the total cotton area [70]. More than 50 distinctive events carrying insect and herbicide resistance genes have been commercially integrated into cotton and cultivated in various regions of the world. After soybean, transgenic cotton is the foremost commercially successful biotech crops. In 1996, Monsanto with Delta and Pineland Co. (D&PL) commercially released two transgenic cotton varieties (NuCOTN[33] and NuCOTN[35]) with a trademark Bollgard™ for general cultivation [71]. Introduction of Bt cotton nearly doubled the lint yield by minimizing production losses due to the infestation of bollworm complex. Cry toxin minimized the use of pesticides by 40%, reduced yield losses by 20%, and enhanced the profitability of farmers by 70% [72]. Currently grown transgenic cotton cultivars constitutively express transgenes resistant to insects/pest attacks. Controlling the spatiotemporal expression of transgenes to the specific plant tissues is a limitation to improve transgenic performance and to decrease its environmental effects. Therefore, use of tissue specific promoter's native to cotton would restrict the transgene expression to certain tissues where it is required, e.g., leaves and stems only. Thus, the identification and utilization of tissue-specific promoters will provide an essential shift in the production of next-generation transgenic cultivars. However, genetically modified crops are setting under intense regulatory formalities which may delay the lab research to come into the market.

In contrast to early genetic engineering techniques that randomly insert a gene of interest into a host genome, genome-editing works with site-specific genetic manipulation in a predefined manner. The genome editing tools have become more accessible for agriculture production over the last decade. The first-generation gene editing tools namely, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR)/Cas system are being deployed to induce site-specific double-strand breaks (DSBs) in plant genomes. CRISPR/Cas9 is one of the emerging tools to mutate the detrimental genes in a pre-defined manner [73]. After the access to *G. hirsutum* genome sequence [74]. CRISPR tools may now be used to achieve predefined DNA modifications. In 2017, Janga et al. reported first successful targeted gene editing study in cotton using CRISPR/Cas9 system [75]. They demonstrated the simultaneous editing of three homeologous genes (*GhPDS*, *GhCLA1*, and *GhEF1*) in the cotton genome. Subsequently, a number of cotton genes have been targeted using CRISPR/Cas9 system like Cloroplastos alterados 1 (*GhCLA1*), vacuolar H+-pyrophosphatase (*GhVP*) [76], cotton fiber development controlling GhMYB25 homoeologous (*GhMYB25-like A* and

GhMYB25-like D) [77], and *Gh14-3-3d* [78] etc. In addition to reported resistance to *Verticillium dahliae* in cotton [78], CRISPR/Cas9 system has also been used to confer resistance against *CLCuD*, a member of family begomoviruses [79–81]. Additionally, already identified several key cotton gene (s) related to seed germination (*GhHSP24*) [82], flowering (*GhAAI66*) [83], fiber development (*GhARF2*, *GhARF18*, *GhXLIM6*) [84,85], gossypol contents (*GhCAD1-A*) [86], resistance to biotic (*GhCRR1*, *GbSOBIR1*) [87,88] and abiotic stresses (*GhABF2*, *GhDsPTP3a*, *GhERF38*) [89–91] can be manipulated.

The delivery of CRISPR reagents into the cotton genome is an imperative step, but one of the most efficient methods of delivering CRISPR/Cas9 into the cotton genome is possible with the shooting apexes as receptors via an *Agrobacterium*-mediated method and DNA-free delivery of CRISPR reagents [92]. Therefore, rational use of CRISPR/Cas9 system in cotton gene editing experiments would be effective for cotton resistance breeding programs against biotic and abiotic stresses. Furthermore, applying this incredible tool to increase lint yield, quality, and climate resilience would have everlasting benefits.

7. Outlook

Revamping of cotton breeding programs by integrating classical breeding approaches, knowledge on functional genomics, and gene editing technologies would open new avenues for selection, characterization, and incorporation of desirable traits in elite cotton cultivars. Tunable and tissue-specific transgene expression and gene stacking can alleviate the potential risks that accompany the resistance against cry genes. In nutshell, the use of CRISPR/Cas system in engineering cotton plants can also be helpful in reducing environmental stresses and disease attacks affecting overall cotton yield and lint quality.

Author Contributions: Conceptualization, M.T.A., S.M. and M.S.M.; writing and draft preparation, M.S.M., S.M. and C.M.; writing—review and editing, M.T.A., S.M., M.S.M., C.M. and X.D.; supervision, X.D. and M.T.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Authors are thankful to Higher Education Commission, Pakistan for provision of research grant under NRPU project "Development of salt tolerance in cotton".

Conflicts of Interest: The authors declare no conflict of interest.

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