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GENOMICS-ASSISTED BREEDING IN COTTON (GOSSYPIUM HIRSUTUM L.) AND GENERATION OF ADVANCE LINES WITH EXCELLENT SEED COTTON YIELD POTENTIAL UNDER CLIMATE CHANGE CONDITIONS

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Abstract

Key message This study found that there was excellent success in pyramiding multiple genetic and genomics sources of climate resilience in advance cotton lines by incorporating the genomics-assisted breeding approach and the developed advance lines showed excellent seed cotton yield under climate change conditions.

Abstract: Climate change, characterized by uncertain weather conditions, has resulted in drastic decrease in cotton (*Gossypium hirsutum* L.) production in major cotton producing countries of the world, including Pakistan. The present research project was designed to develop cotton advance lines with excellent performance potential under climate change conditions by incorporating a genomics-assisted breeding approach. A comprehensive cotton germplasm consisting of forty accessions was evaluated to select suitable parents for crossing purposes. The progenies in the successive generations were evaluated on the basis of morphological, biochemical, seed cotton yield and molecular (presence of desirable band of *KCS6* gene and simple sequence repeat markers, NAU2083, NAU2540, NAU3901). Two elite cotton lines, SI-1 and SI-2, were developed. In terms of seed cotton yield per plant, SI-1 showed an increase of 61% and 87% to the commercial check in 2021 and 2022, respectively; and SI-2 manifested an increase of 51% and 82% to the commercial check during 2021 and 2022, respectively. These results indicated that genomics-assisted breeding approach had promising success in developing elite cotton lines with excellent yield potential under climate change conditions, and, thus, it should be incorporated as a routine tool in breeding programs for rapid development of elite crop cultivars in view of climatic uncertainty.

Introduction

Pakistan is among the countries badly affected by climate change, characterized by weather uncertainty. Climate change has resulted in frequent dry spells, heat stress and sudden torrent rains during cotton growth period in major cotton regions of the world, including Pakistan (Arshad et al. 2021; Saeed et al. 2024). This multitude of climatic conditions demand for the development of cotton cultivars with sustainable yield potential under climate change conditions. Extreme environmental conditions, such as drought, heat stress, and torrent rains have an impact on cotton growth, productivity, and fibre quality (Parida et al. 2008; Saeed et al. 2024). Drought and heat stress have significant impact on agricultural output, root proliferation systems, plant diseases, and

insect attack; and resulted in a 34% decline in cotton production in Pakistan (Ullah et al. 2017; Zahid et al. 2019). Drought stress has an impact on plant physiology through cellular and molecular pathways (Li 2019). There are four types of drought tolerance mechanisms in plants: drought avoidance, drought tolerance, drought recovery, and drought escape (Wu et al. 2015). Drought avoidance and drought tolerance are the two major strategies used by plants to combat drought stress. In terms of drought tolerance, the cotton crop's unpredictable behavior is limited (Saeed et al. 2011). At the seedling stage, the tolerance variability is available. To work on the development of drought-tolerant genotypes, it's critical to understand how plants respond and behave. Drought sensitivity and tolerance in upland cotton could be classified using a variety of morphological features (Jaleel et al., 2009). Significant differences in morphological parameters such as shoot length (SL), number of bolls per plant, plant height, root length (RL), and boll weight was recorded (Zahid et al. 2021). Root shape is important in determining drought response. Drought stress lowered photosynthesis due to damage to the photosynthetic machinery, resulting in early leaf ageing, a decrease in leaf growth and surface area, and a reduction in food production (Garrido et al. 2023; Wahid et al. 2005). Drought also causes the plant's stomata to close, reducing CO2 uptake and making the plant vulnerable to solar damage (Baytar et al. 2018). Relative water content (RWC) is a selection criterion for drought-tolerant genotypes in cotton seedlings. Cotton is more vulnerable to low water potential for photosynthesis than stomatal conductance, which is owing to a loss in photosynthetic enzyme synthesis and activity during drought (Yang et al. 2016). Drought stress reduces the stability of cell membranes, chlorophyll a and b levels, dry matter stocking, and RWC in cotton. Studies of cotton genotypes under various levels of water stress revealed that when drought stress increases, the water content of the leaf and the quantum yield of photosystem-II drop (Wang et al. 2007). Water scarcity impairs cellular growth and inhibits leaf and stem elongation, and reduces the quantity of floral buds (Wang et al. 2007). Drought disrupts the balance of reactive oxygen species (ROS) and antioxidant production, which can lead to ROS accumulation in plant systems (Reddy et al. 2004). In Pakistan, cotton is exposed to high temperature stress in the field as during cotton growth period temperature goes beyond 40 °C (Zahid et al. 2016). This high temperature stress leads to excessive squares, flowers and young bolls shedding (Farooq et al. 2023; Zahid et al. 2016). Similarly, high moisture conditions lead to excessive vegetative growth and reduce seed cotton yield (Kumar et al. 2023).

Cuticular wax, a major component of plant cuticle, is reported to impart tolerance to various biotic and abiotic stresses including drought stress. Cuticular wax, epi- and intra-cuticular wax, consists of a complex mixture of very-long-chain (VLC) aliphatic compounds, triterpenoids and minor metabolites such as sterols and flavonoids extractable by rapid immersion in organic solvents (Samuels et al. 2008). Very-long-chain fatty acids are converted to (i) primary alcohols and alkyl esters through alcohol-forming pathway; and (ii) aldehydes, alkanes, secondary alcohols, ketones, sterols, and δ-amyrin through alkane-forming pathway (Bernard and Joubès 2013; Yeats and Rose 2013). Total wax contains 17–18% of primary alcohols and alkyl esters. Total wax consists of 12–14% of primary alcohols only. Alkyls esters, with chain-length ranging from C_{38} to C_{48}, are only minor components in Arabidopsis (<5%). In Arabidopsis, aldehydes, alkanes, secondary alcohols and ketones account for more than 80% of the total wax amounts, with alkanes making up to 70% of the total waxes in rosette leaves. Cuticular wax biosynthesis is very complex. Cuticular wax biosynthesis takes place in the endoplasmic reticulum of epidermal cells from the fatty acids derived from leucoplasts. Various steps in the biosynthesis of cuticular wax include a) activation of fatty acids; b) fatty acids elongation; c) synthesis of cuticular wax compounds; and d) transport of cuticular wax compounds to the place of final deposition (cuticle). Fatty acid elongation is accomplished in four successive enzymatic reactions. These reactions are condensation, reduction, dehydration, and reduction (Lessire et al. 1998). Multiple elongase complexes are reported in plants with distinct chain-length specificity which may perform sequential and/or parallel reactions to produce the broad chain-length-range of VLCFAs (Millar and Kunst 1997). In Arabidopsis, six KCS (*KCS1*, *KCS2*, *KCS10/FDH*, *KCS13/HIC*, *KCS20*, and *KCS6/CER6*) were found to have role in

cuticle compounds synthesis (Lee and Suh 2015; Rahman et al. 2021). Among these, *CER6*/*KCS6* is the only gene referred as strictly wax-specific (Huang et al. 2022, 2023). *KCS6* is the key gene having role in abiotic stress tolerance in plants (Guo et al. 2020). Based on the previous findings, *KCS6* gene was selected for the present genomics-assisted breeding (GAB) approach to develop elite climate smart cotton lines. Additionally, three SSR markers, NAU2083, NAU, 2540, NAU3901 were also used for the marker-assisted selection (MAS) in segregating generations. These markers were reported to have associations with abiotic stress tolerance in cotton (Saeed et al. 2011, 2014). This study aimed to develop elite advance cotton lines with better yield potential under climate change conditions by pyramiding multiple gene/allele sources through genomics-assisted breeding approach ensuring sustainable cotton production.

Materials and methods

Plant Material, germplasm screening and performance evaluation

Forty cotton genotypes were collected from exotic and indigenous sources (Table S1). Screening of cotton germplasm, to select suitable parents for crossing, was performed in the research field of Government College University Faisalabad (GCUF), New Campus, Jhang Road, Faisalabad 38000, Pakistan during 2018. This screening was done on the basis of molecular, biochemical, and morphological attributes (Table S2). On the basis of this evaluation, ten genotypes were selected for making the crosses (Table S3). Planned crosses were performed between the selected parents (Table S4). From these crosses generations were developed from F_2 up to F_6. Selection of suitable lines was done in every generation on the basis of selection criteria (Table S2). To save time, F_1, F_3, and F_5 lines were evaluated in winter nurseries. During November 2018, The F_0 seed of 10 crosses was sown in green house for generation advancement. Selfing of the F_1 plants was carried out. From these F_1 plants seed obtained for the sowing of F_2 generation in the field. Ten F_2 progenies of the crosses were sown in the field during April 2019. From these F_2 progenies, selection of 25 single plants per progeny on the basis of molecular, biochemical, and morphological attributes (Table S2) was made and finally 5 plants per progeny were selected on the basis of fiber traits. Sowing for raising of F_3 progenies in greenhouse was carried out on 13-11-2019. From F_3 progenies, selection of 25 progenies and 5 single plant per progeny on the basis of desirable traits was made. Harvesting of F_3 progenies was done on 20-03-2020. Sowing for raising of 125 F_4 progenies in field was carried out on 20-04-2020. From 125 F_4 progenies, selection of 20 progenies and 5 single plants per progeny on the basis of desirable traits was made. Sowing for raising of 100 F_5 progenies in greenhouse was carried out on 11-11-2020. From 100 F_5 progenies, selection of 10 progenies and 5 single plants per progeny on the basis of desirable traits was made. Harvesting of F_5 progenies was done on 20-03-2021. Sowing for raising of 50 F_6 progenies in field was carried out on 02-06-2021. From 50 F_6 progenies, two promising progenies were selected with excellent seed cotton yield potential under climate change conditions. Their seed was bulked and these advance lines were designated as SI-1 and SI-2, respectively. Seed of these advance lines was also sown next year in the field on 19-05-2022 for their second year performance evaluation.

Cotton germplasm, progenies and the advance lines were evaluated/ screened on the basis of molecular (presence of desirable band of *KCS6* gene and three simple sequence repeats (SSR) markers (NAU2083, NAU2540, and NAU3901); cotton leaf curl virus disease index (DI, %); morphological parameters i.e., number of sympodial branches per plant (SB), number of monopodial branches per plant (MB), plant height (PH); yield i.e., number of bolls per plant (BP), boll weight (BW, g), seed cotton yield per plant (SCY, g), ginning out turn (GOT, %); fiber quality i.e., staple length (SL, mm), micronaire (MIC, µg/in), fiber strength (FS, g/tex); and biochemical i.e., cuticular wax content (WC).

Statistical analysis of the phenotypic data

The analysis of the phenotypic data was performed using Microsoft Excel, Origin (https://www.originlab.com/index.aspx?go=PRODUCTS/Origin) and CoStat v. 6.303. The figures

for descriptive statistics were drawn in Microsoft Excel and the correlogram for Pearson' correlation coefficients were drawn in Origin. CoStat v. 6.303 was used to assess analysis of variance (ANOVA) estimates. ANOVA was performed according to RCBD with three replications and one factor (varieties), as well as, with two factors (varieties and years).

Genotyping of advance lines and commercial check with *KCS6* gene and simple sequence repeats (SSR) markers

For genotyping of advance lines and the commercial check with *KCS6* gene and SSR markers, the DNA from young leaves of each genotype was extracted by following the CTAB method (Paterson et al. 1993). Quantification of the DNA was carried out by NanoDrop™ 2000 Spectrophotometer, Thermo Scientific™, USA. DNA of the advance lines and commercial check was amplified using primers of *KCS6* gene and three SSR markers, NAU2083, NAU2540, and NAU3901. DNA was amplified using primers in a PCR reaction solution with a final volume of 10 µl. DreamTaq Green PCR Master Mix (2X), Thermo Scientific™, USA was used to prepare the PCR reaction solution with the composition: 5 µl DreamTaq Green PCR Master Mix (2X); 0.5 µM forward primer; 0.5 µM reverse primer; 10 ng template DNA; nuclease-free water (to make total volume of 10 µl). The program for the PCR reaction was set at 95ºC for 3 minutes; 30 cycles of (94ºC for 30 seconds, 50-57ºC for 30 seconds, 72ºC for 45 seconds); 72ºC for 5 minutes; and 4ºC for 10 minutes. PCR products were separated through polyacrylamide gel electrophoresis, stained with silver staining and visualized by gel documentation system. PCR product bands were recorded according to the sizes in bp (base pairs).

Results

Screening of parents, performance evaluation and molecular characterization of progenies Forty cotton genotypes/lines collected from indigenous and exotic sources were planted at research field, Government College University Faisalabad (GCUF), New Campus, Jhang Road, Faisalabad, Pakistan. On the basis of molecular, biochemical, morphological, and yield attributes, 10 parents were selected for crossing program.

Parents showed considerable genetic diversity with regard to the biochemical, morphological, and yield attributes (Table S5). Ten crosses were attempted for generation advancement (Table S4). Seeds of these crosses were sown in the greenhouse to raise F_1 progenies.

The detail of the performance for the studied traits of the generations from F_1 to F_6 are elaborated in Table S6 and Figure 1. These results indicated that the progenies were highly diverse.

In F_1, the mean DI was 0.67%. The minimum and maximum value of DI was 0.3% and 1.73%, respectively. In F_2, the mean DI was 0.36%. The minimum and maximum value of DI was 0% and 1.37%, respectively. In F_3, the mean DI was 0.24%. The minimum and maximum value of DI was 0% and 0.7%, respectively. In F_4, the mean DI was 0.39%. The minimum and maximum value of DI was 0% and 0.78%, respectively. In F_5, the mean DI was 0.46%. The minimum and maximum value of DI was 0% and 0.77%, respectively. In F_6, the mean DI was 0.44%. The minimum and maximum value of DI was 0% and 0.88%, respectively.

In F_1, the mean SB was 20.73. The minimum and maximum value of SB was 15 and 25, respectively. In F_2, the mean SB was 21.37. The minimum and maximum value of SB was 15 and 27, respectively. In F_3, the mean SB was 23.55. The minimum and maximum value of SB was 21.67 and 26.68, respectively. In F_4, the mean SB was 25.00. The minimum and maximum value of SB was 21.67 and 27.33 respectively. In F_5, the mean SB was 23.62. The minimum and maximum value of SB was 20 and 25.67, respectively. In F_6, the mean SB was 25.34. The minimum and maximum value of SB was 21.5 and 28, respectively.

In F_1, the mean MB was 1.41. The minimum and maximum value of MB was 0.67 and 2.33, respectively. In F_2, the mean MB was 1.16. The minimum and maximum value of MB was 0.33 and 2.67, respectively. In F_3, the mean MB 0.50. The minimum and maximum value of MB was 0 and 1, respectively. In F_4, the mean MB was 1.07. The minimum and maximum value of MB was 0 and 2,

respectively. In F_5, the mean MB was 0.99. The minimum and maximum value of MB was0.33 and 0.33, respectively. In F_6, the mean MB was 0.74. The minimum and maximum value of MB was 0.25 and 1, respectively.

In F_1, the mean PH was 104.63 cm. The minimum and maximum value of PH was 75 cm and 140 cm, respectively. In F_2, the mean PH was 72.28 cm. The minimum and maximum value of PH was 63.33cm and 84 cm, respectively. In F_3, the mean PH was 88.56cm. The minimum and maximum value of PH was 75.33 cm and 101 cm, respectively. In F_4, the mean PH was 78.58 cm. The minimum and maximum value of PH was 58 cm and 99.33 cm, respectively. In F_5, the mean PH was 84.80 cm. The minimum and maximum value of PH was 76 cm and 91.33 cm, respectively. In F_6, the mean PH was 84.99 cm. The minimum and maximum value of PH was 71 cm and 97.33 cm, respectively.

In F_1, the mean BP was 49.53. The minimum and maximum value of BP was 25 and 78, respectively. In F_2, the mean BP was 27.80. The minimum and maximum value of BP was 21.67 and 34.67, respectively. In F_3, the mean BP was 23.63. The minimum and maximum value of BP was 21.67 and 26.68, respectively. In F_4, the mean BP was 25.48. The minimum and maximum value of BP was 21.67 and 29.67, respectively. In F_5, the mean BP was 25.26. The minimum and maximum value of BP was 21 and 29.33, respectively. In F_6, the mean BP was 25.93. The minimum and maximum value of BP was 21.33 and 29.33, respectively.

In F_1, the mean BW was 3.15 g. The minimum and maximum value of BW was 2 g and 4.3 g, respectively. In F_2, the mean BW was 3.31 g. The minimum and maximum value of BW was 1.75 g and 4.5 g, respectively. In F_3, the mean BW was 3.41g. The minimum and maximum value of BW was 2.5 g and 4.4 g, respectively. In F_4, the mean BW was 3.20 g. The minimum and maximum value of BW was 2.27 g and 3.63 g, respectively. In F_5, the mean BW was 3.13 g. The minimum and maximum value of BW was 2.2 g and 3.47 g, respectively. In F_6, the mean BW was 3.25 g. The minimum and maximum value of BW was 2.2 g and 3.67 g, respectively.

In F_1, the mean SCY was 155.5 g. The minimum and maximum value of SCY was 73.5g and 254.6 g, respectively. In F_2, the mean SCY was 92.40 g. The minimum and maximum value of SCY was 51.92g and 131.15 g, respectively. In F_3, the mean SCY was 80.47 g. The minimum and maximum value of SCY was 55.63 g and 96.9 g, respectively. In F_4, the mean SCY was 82.46 g. The minimum and maximum value of SCY was 60.38 g and 99.73 g, respectively. In F_5, the mean SCY was 79.91 g. The minimum and maximum value of SCY was 46.2 g and 99.73 g, respectively. In F_6, the mean SCY was 82.13g. The minimum and maximum value of SCY was 63.8g and 100.52 g, respectively.

In F_1, the mean GOT was 35.47%. The minimum and maximum value of GOT was32% and 40%, respectively. In F_2, the mean GOT was 38.05 %. The minimum and maximum value of GOT was 35.67 % and 45.5 %, respectively. In F_3, the mean GOT was 37.92 %. The minimum and maximum value of GOT was 36 % and 40.33 %, respectively. In F_4, the mean GOT was 37.6 %. The minimum and maximum value of GOT was 36 % and 39.5 %, respectively. In F_5, the mean GOT was 37.49 %. The minimum and maximum value of GOT was 33.66% and 39.75 %, respectively. In F_6, the mean GOT was 38.09 %. The minimum and maximum value of GOT was 36.33 % and 39.67 %, respectively.

In F_1, the mean SL was 25.63 (mm). The minimum and maximum value of SL was 21(mm) and 30(mm), respectively. In F_2, the mean SL was 26.65 (mm). The minimum and maximum value of SL was 21.45(mm) and 28.68(mm), respectively. In F_3, the mean SL was 27.98 (mm). The minimum and maximum value of SL was 26.5(mm) and 28.93 (mm), respectively. In F_4, the mean SL was 27.65(mm). The minimum and maximum value of SL was 25.83 (mm) and 29.77(mm), respectively. In F_5, the mean SL was 27.26(mm). The minimum and maximum value of SL was 25.5 (mm) and 28.93mm), respectively. In F_6, the mean SL was 27.74(mm). The minimum and maximum value of SL was 25.3(mm) and 28.92(mm), respectively.

In F_1, the mean MIC was 4.41(µg/in).The minimum and maximum value of MIC was 3 (µg/in) and 5.5 (µg/in), respectively. In F_2, the mean MIC was 4.24 (µg/in).The minimum and maximum value

of MIC was 2.8 (µg/in) and 4.75 (µg/in), respectively. In F_3, the mean MIC was 4.31(µg/in). The minimum and maximum value of MIC was 3.47 (µg/in) and 4.8 (µg/in), respectively. In F_4, the mean MIC was 4.19(µg/in). The minimum and maximum value of MIC was 3.13 (µg/in) and 5(µg/in), respectively. In F_5, the mean MIC was 4.41 (µg/in).The minimum and maximum value of MIC was 3.5 (µg/in) and 4.73 (µg/in), respectively. In F_6, the mean MIC was 4.38 (µg/in). The minimum and maximum value of MIC was 3.37 (µg/in) and 4.73 (µg/in), respectively.

In F_1, the mean FS was 27.4 (g/tex). The minimum and maximum value of FS was 23 (g/tex) and 32 (g/tex), respectively. In F_2, the mean FS was 27.02 (g/tex). The minimum and maximum value of FS was 25.15 (g/tex) and 29.7 (g/tex), respectively. In F_3, the mean FS was 26.93 (g/tex). The minimum and maximum value of FS was 26 (g/tex) and 27.63 (g/tex), respectively. In F_4, the mean FS was 26.64 (g/tex). The minimum and maximum value of FS was 24.73 (g/tex) and 28.23 (g/tex), respectively. In F_5, the mean FS was 28.01 (g/tex). The minimum and maximum value of FS was 23 (g/tex) and 32 (g/tex), respectively. In F_6, the mean FS was 28.63 (g/tex). The minimum and maximum value of FS was 26.33 (g/tex) and 31 (g/tex), respectively.

In F_1, the mean WC was 52.79 (µg /cm^2). The minimum and maximum value of WC was 31.5 (µg /cm^2) and 61.67 (µg /cm^2), respectively. In F_2, the mean WC was 53.59 (µg /cm^2). The minimum and maximum value of WC was 45.73 (µg /cm^2) and 58.98 (µg /cm^2), respectively. In F_3, the mean WC was 52.50 (µg /cm^2) .The minimum and maximum value of WC was 43.83 (µg /cm^2) and 57.8 (µg /cm^2), respectively. In F_4, the mean WC was 60.86 (µg /cm^2) .The minimum and maximum value of WC was 50.43 (µg /cm^2) and 66.8 (µg /cm^2, respectively. In F_5, the mean WC was 61.45 (µg /cm^2) .The minimum and maximum value of WC was 56.5 (µg /cm^2) and 65.9 (µg /cm^2), respectively. In F_6, the mean WC was 60.97 (µg /cm^2) .The minimum and maximum value of WC was 47.5 (µg /cm^2) and 70 (µg /cm^2), respectively.

In every generation, the selection was also done on the basis of the desirable band of *KCS6* gene and the SSR markers NAU2540, NAU2083, and NAU3901 (Figure 2). *KCS6* is the gene involved in the cuticular wax biosynthesis pathway in plants. Whereas, SSR NAU2540, NAU2083, and NAU3901 markers were found to be associated with drought tolerance in cotton (Saeed et al. 2011).

ANOVA for studied traits

In F_1, there were marked differences among cotton progenies for all the studied traits (Table 1). The cotton progenies had significant differences ($P < 0.05$) for MB. The cotton progenies had highly significant differences ($P < 0.01$) for PH, GOT, MIC, and FS. The cotton progenies had very high significant differences ($P < 0.001$) for DI (%), SB, BP, BW, SCY, SL, and WC.

In F_2, there were marked differences among cotton progenies for all the studied traits (Table 1). The cotton progenies had significant differences ($P < 0.05$) for DI and PH. The cotton progenies had highly significant differences ($P < 0.01$) for BP, GOT, FS, and WC. The cotton progenies had very high significant differences ($P < 0.001$) for DI (%), SB, MB, BW, SCY, SL, and MIC.

In F_3, there were marked differences among cotton progenies for all the studied traits, except SB (Table 1). The cotton progenies had significant differences ($P < 0.05$) for D1, MB, PH and SL. The cotton progenies had highly significant differences ($P < 0.01$) for GOT and FS. The cotton progenies had very high significant differences ($P < 0.001$) for BP, BW, SCY, MIC, and WC.

In F_4, there were marked differences among cotton progenies for all the studied traits (Table 1). The cotton progenies had highly significant differences ($P < 0.01$) for DI (%), SB, PH, and GOT. The cotton progenies had very high significant differences ($P < 0.001$) for MB, BP, BW, SCY, SL, MIC, FS and WC.

In F_5, there were marked differences among cotton progenies for all the studied traits (Table 1). The cotton progenies had significant differences ($P < 0.05$) for DI (%), PH, FS and WC. The cotton progenies had highly significant differences ($P < 0.01$) for SB, MB, and MIC. The cotton progenies had very high significant differences ($P < 0.001$) for BP, BW, SCY, GOT, and SL.

In F_6, there were marked differences among cotton progenies for all the studied traits (Table 1). The cotton progenies had significant differences ($P < 0.05$) for MB and SL. The cotton progenies had

highly significant differences ($P < 0.01$) for DI (%), BP, BW, SCY and GOT. The cotton progenies had very high significant differences ($P < 0.001$) for SB, PH, MIC, FS and WC.

Characterization of cotton advance lines, SI-1 and SI-2 and commercial check for yield and biochemical traits

Two elite advance lines, SI-1 and SI-2, had excellent yield potential compared to the commercial check variety, FH-Lalazar. In terms of BW, SI-1 showed an increase of 70% and 42% in 2021 and 2022, respectively. Similarly, SI-2 manifested an increase of 60% and 42% in BW during 2021 and 2022, respectively. In terms of SCY, SI-1 showed an increase of 61% and 87% in 2021 and 2022, respectively; and SI-2 manifested and increase of 51% and 82% in SCY during 2021 and 2022, respectively. These results indicated that genomics-assisted breeding approach had greater success in developing elite cotton lines with excellent yield potential under climate change conditions.

Two advance lines were highly diverse in biochemical parameters (Table S7). In both 2021 and 2022, BW and SCY had positive correlation with all studied biochemical traits, except MDA (Table 3). It showed that the advance lines, SI-1 and SI-2, accumulated favor genes/alleles involved in coding for the biochemical attributes ensuring drought tolerance i.e., increase in the amount of Chl a, Chl b, CRT, TSP, TFA, PHN, HPO, POD, and CAT and decrease in the amount of MDA.

Genotyping of cotton advance lines, SI-1 and SI-2 and commercial check with *KCS6* gene and SSR primers

Two advance lines, SI-1 and SI-2, showed the desirable band of *KCS6* gene, as well as, bands of SSR markers, NAU2540, NAU2083, and NAU3901. It showed that the genomics-assisted breeding approach was successful in pyramiding the desirable genes in the advance lines. This pyramiding of targeted genes/alleles was manifested in the better sustainable SCY in advance lines under climate change conditions.

Discussion

Climate change is characterized by uncertain weather conditions. For sustainable production under climate change scenario, crop cultivars would have to possess multiple sources of climate resilience. The GAB approach has capacity to develop elite climate smart crop cultivars in shortest possible time. In the present research, GAB was incorporated to develop climate smart advance cotton lines. For genomics-assisted selection (GAS), *KCS6* gene and three SSR markers, NAU2083, NAU2540, and NAU3901 were used. In the successive segregating generations, to make the selection more robust DI, SB, MB, PH, BP, BW, SCY, GOT, SL, MIC, FS, WC were also used.

Plant architecture has great importance in sustainable cotton production under climate change conditions. The cotton cultivars with optimum plant height potential had better seed cotton yield (Saeed et al. 2011, 2024). It is of prime concern that under frequent heavy rains those cotton genotypes perform better which have better balance between the vegetative and reproductive growth. The genotypes which continued a robust vegetative growth under heavy rains did not show good seed cotton yield (Yan et al. 2019). Similarly, MB and SB are also key attributes for SCY. SSR markers NAU 2083, located on chromosome 26 (D12), and NAU 2540, located on chromosome 20 (D10), were associated with water-deficit tolerance in cotton and controlled osmotic adjustment and osmotic potential, respectively. Whereas, SSR marker NAU 3901, located on chromosome 15 (D1), was associated with PH under water-deficit conditions (Saeed et al. 2011). MAS based on NAU 2083, NAU 2540 and NAU 3901 helped to pyramid genetic loci ensuring a plant architecture favoring sustainable excellent SCY under climate change conditions.

BP is an important trait to determine seed cotton yield. Marked decrease in BP was noticed under water deficit and heat stress conditions (Pettigrew 2004, 2008). Other yield-related traits such BW and SCY had similar trend under adverse environmental conditions. Cotton genotypes under water stress conditions recorded a significant decline in BW as compared to well-watered condition (Saeed et al. 2011). GOT is a polygenic trait and highly influenced by the environment. Cotton

genotypes with higher GOT are preferred by the ginners. Mahmood et al. (2006), Osborne et al. (2006) and Karademir et al. (2009) reported that GOT was remarkably reduced due to water stress. Increase in SCY is ultimate objective of plant breeders. This trait is highly influenced by the environmental factors. In the present research, as a result of GAB a number of favorable genetic loci were pyramided in the two advance lines, SI-1 and SI-2. The resulted in the tremendous increase in the yield-related traits i.e., BP, BW and SCY and, thus, the developed advance lines surpassed the commercial check variety for consecutive two years under field experimentation.

The use of *KCS6* gene in the GAS contributed significantly in pyramiding multiple genetic and genomic sources of climate resilience in the advance lines which were manifested in the better values for various studied traits of seed cotton yield and fiber quality. *KCS6* is the major gene in cuticular wax biosynthesis. Cuticular wax was reported for tolerance against environmental adversities such as drought, heat stress and biotic factors in several crop plants (Khoudi et al. 2023; Saeed et al. 2018, 2024; Trivedi et al. 2019; Xue et al. 2017). Guo et al. (2016) reported that cuticular wax was involved in drought tolerance in wheat and cuticular wax content could be a good selection criterion for screening wheat genotypes for drought tolerance. Cuticular waxiness resulted in 2%–50% reduction in transpiration (Sultana et al. 2014). Presence of cuticular wax on leaves had been used as criteria for abiotic stress tolerance in chickpea (*Cicer arietinum* L.) and pigeonpea [*Cajanus cajan* L. (Millsp.)] (Choudhary et al. 2018). There was increased wax deposition in drought tolerant ryegrass line (Pan et al. 2017). There were reports in number of other plant species that cuticular wax protected plants against drought stress (Saeed et al. 2024; Schuster et al. 2016; Xue et al. 2017; Zeisler and Schreiber 2016; Zeisler-Diehl et al. 2018). Thus, presence of adequate amount of cuticular wax on leaf surfaces was condusive to increase drought tolerance, as well as, it protected plants against other environmental adversities. Presence of cuticular wax sheltered plants against the damaging effect of high and low temperatures (Joubès et al. 2008; Mondal 2011; Shepherd and Griffiths 2006). In the present research, the developed advance lines, SI-1 and SI-2, had excellent climate resilience and, thus, the GAB approach was successful in pyramiding multiple genetic and genomic sources of resistance to environmental adversities. This research will open new horizons for GAB approach in future.

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Author contribution statement SA performed the research work and wrote manuscript; MS conceived the project, designed the experiments, analysed the results, and edited manuscript; MSMC analysed data and edited manuscript. All authors read the manuscript and approved the final manuscript.

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Data availability All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Declarations

Conflict of interest: The authors declare that they have no conflicts of interest associated with this work.

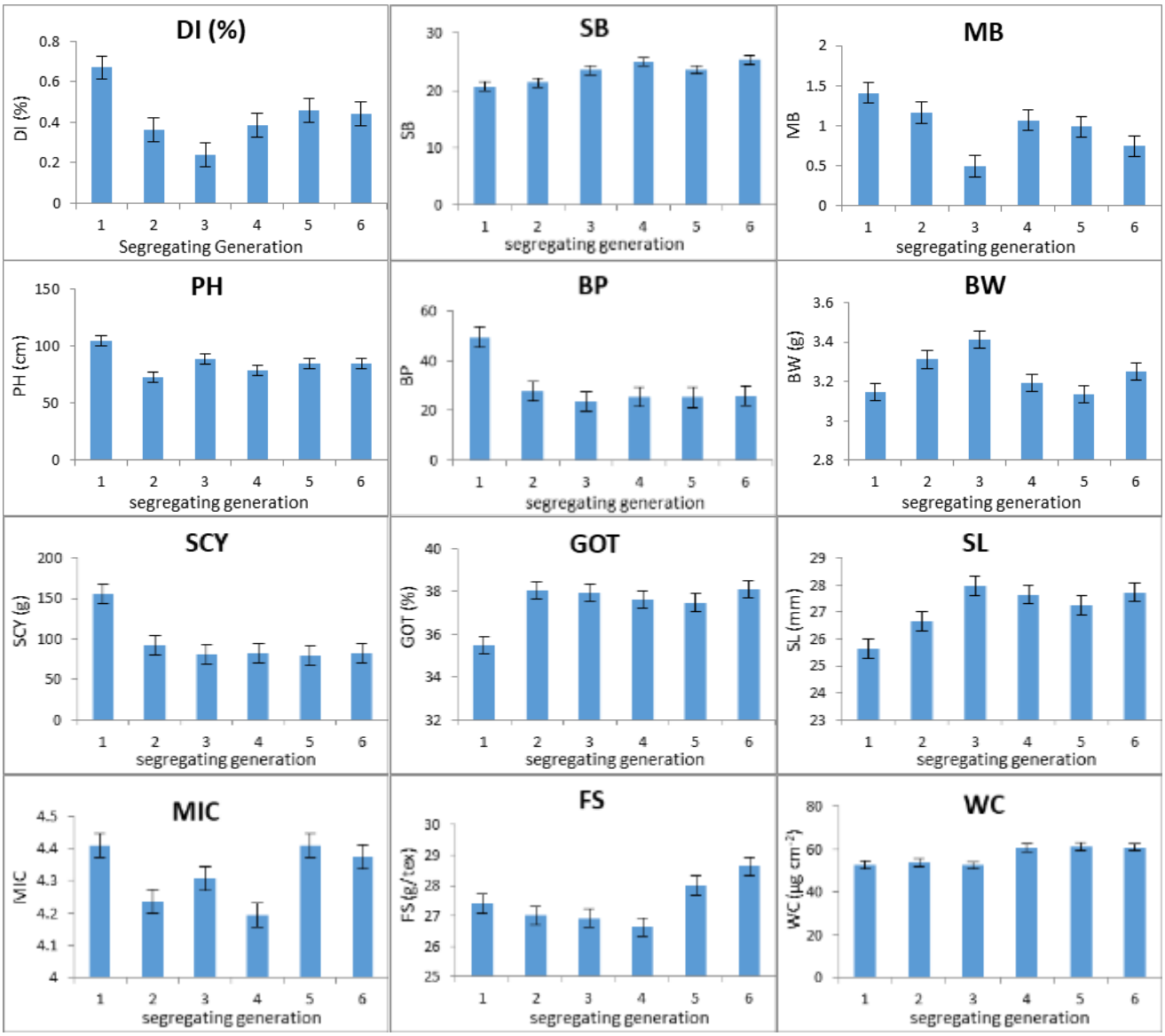
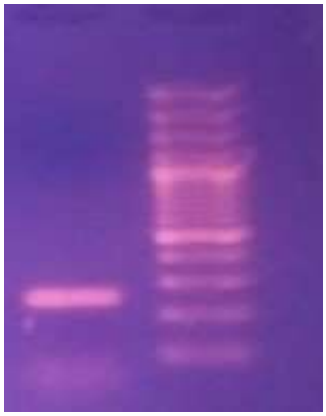


Figure 1. Performance of the progenies with respect to the studied traits in F_1-F_6.



A **B**

Figure 2. Genotyping with *KCS6* primer. Agarose gel of PCR product; A. *KCS6* band and B. 100 bp plus DNA ladder.

Table 1. Mean squares of ANOVA for the studied traits in F_1-F_6.

SoV/Trait	Generation	Blocks	Genotype	Error	R^2	CoV (%)
DI (%)	F1	0.0533 ns	0.3469 ***	0.0169	0.914	19.3848
	F2	0.2499 ns	0.1851 *	0.0735	0.6207	74.4135
	F3	0.0034 ns	0.1641 *	0.0274	0.751	69.3061
	F4	0.9942 ns	10.9298 **	0.021	0.851	37.4479
	F5	0.0528 ns	0.1269 *	0.0212	0.7825	31.8054
	F6	0.0040 ns	0.1878 **	0.0242	0.7961	35.3069
SB	F1	0.4333 ns	20.0592 ***	3.3592	0.75	8.84
	F2	2.5333 ns	25.5149 ***	2.6814	0.8294	7.664
	F3	4.2875 ns	1.5942 ns	1.2887	0.5919	4.8203
	F4	3.4838 ns	6.8650 **	0.8275	0.8388	3.638
	F5	1.6074 ns	5.6962 **	0.656	0.832	3.4288
	F6	0.6893 ns	13.8314 ***	0.6662	0.914	3.2211
MB	F1	0.0737 ns	0.2390 *	0.0861	0.597	20.8768
	F2	0.2311 ns	1.0847 ***	0.1051	0.8439	27.98
	F3	0.04701 ns	0.2030 *	0.0331	0.7737	36.9783
	F4	0.0222 ns	1.1394 ***	0.063	0.9013	23.5345
	F5	0.0263 ns	0.7658 **	0.0797	0.8302	28.697
	F6	0.0421 ns	0.1579 *	0.0277	0.7639	22.3413
PH (cm)	F1	126.5333 ns	850.2555 **	161.422	0.7312	12.1425
	F2	0.7975 ns	63.4468 *	21.9615	0.5915	6.484
	F3	3.6740 ns	138.2175 *	24.4981	0.7409	5.5892
	F4	24.1408 ns	419.4993 **	34.0562	0.8637	7.4268
	F5	21.2171 ns	43.5192 *	7.0957	0.7922	3.1414
	F6	0.7030 ns	205.6004***	5.9459	0.9454	2.8691
BP	F1	70.2333 ns	488.0149***	29.6038	0.8949	10.9843
	F2	0.3210 ns	21.6459 **	5.161	0.6779	8.1711
	F3	0.1729 ns	10.7933 ***	0.2341	0.9588	2.0475
	F4	1.9950 ns	22.9814 ***	1.4231	0.8939	4.6833
	F5	1.4338 ns	16.9359 ***	0.715	0.925	3.3474
	F6	3.4950 ns	16.6442 **	1.2275	0.8822	4.2732
BW	F1	0.0103 ns	1.0180 ***	0.1819	0.7372	13.5508

	F2	0.0899 ns	0.8964 ***	0.104	0.8151	9.7352
	F3	0.0127 ns	0.9407 ***	0.0244	0.951	4.5777
	F4	7.8241 ns	0.6635 ***	0.0132	0.9617	3.605
	F5	0.0069 ns	0.7184 ***	0.0032	0.9911	1.8078
	F6	0.0175 ns	0.5027 **	0.0535	0.8268	7.1211
SCY (g)	F1	300.513 ns	9180.998***	609.876	0.8834	15.8814
	F2	92.4227 ns	1188.6869***	89.0673	0.8717	10.2135
	F3	15.1264 ns	462.4617 ***	15.6962	0.9373	4.9233
	F4	45.6374 ns	465.7398 ***	15.9581	0.9387	4.8448
	F5	35.4558 ns	930.8269 ***	15.1773	0.969	4.8752
	F6	60.0738 ns	242.9885 **	26.326	0.8383	6.247
GOT (%)	F1	2.6333 ns	7.6444 **	1.5222	0.7299	3.4788
	F2	1.9387 ns	10.8334 **	1.9611	0.7418	3.6805
	F3	0.6351 ns	2.9460**	0.4002	0.803	1.6682
	F4	0.1555 ns	3.7658 **	0.424	0.8192	1.732
	F5	0.1758 ns	9.1509 ***	0.4672	0.9081	1.8235
	F6	0.9430 ns	1.6679 **	0.2359	0.8193	1.2752
SL (mm)	F1	3.3333 ns	16.9962 ***	1.963	0.8188	5.4658
	F2	1.004 ns	5.8682 ***	0.8899	0.7739	3.5395
	F3	0.5748 ns	1.2708 *	0.1932	0.8012	1.571
	F4	0.1810 ns	3.4932 ***	0.167	0.9148	1.4777
	F5	0.1534 ns	4.9918 ***	0.0942	0.9641	1.126
	F6	0.0683 ns	2.2972 *	0.353	0.7675	2.1421
MIC (µg/in)	F1	0.289 ns	0.8755 **	0.2182	0.6829	10.5938
	F2	0.0644 ns	0.7222 ***	0.0699	0.8404	6.2399
	F3	0.0094 ns	0.5634 ***	0.019	0.9375	3.194
	F4	0.0408 ns	0.9013 ***	0.026	0.9467	3.8419
	F5	0.0583 ns	0.3258 **	0.0457	0.7954	4.8443
	F6	0.0142 ns	0.7199 ***	0.008	0.9785	2.039
FS (g/tex)	F1	0.4 ns	18.2074 **	4.0297	0.6942	7.3262
	F2	0.6445 ns	2.3229 **	0.5337	0.698	2.704
	F3	0.1093 ns	0.4483 **	0.0637	0.7981	0.9366

	F4	0.2460 ns	4.2603 ***	0.0884	0.9611	1.1169
	F5	3.2351 ns	12.6778 *	3.0778	0.699	6.263
	F6	0.5167 ns	7.2562 ***	0.3743	0.9094	2.1367
WC (µg/cm^2)	F1	4.7560 ns	152.1492 ***	12.1534	0.863	6.604
	F2	17.7730 ns	25.6781 **	6.493	0.6952	4.7553
	F3	6.0392 ns	43.0753 ***	2.1738	0.9139	2.8088
	F4	2.8421 ns	93.8840 ***	2.2191	0.9555	2.4478
	F5	2.1444 ns	20.1620 *	4.2523	0.714	3.3557
	F6	10.9729 ns	126.9090 ***	4.1459	0.941	3.3395

DI Cotton leaf curl virus disease index; *SB* number of sympodial branches per plant; *MB* number of monopodial branches per plant; *PH* plant height: *BP* number of bolls per plant; *BW* boll weight; *SCY* seed cotton yield per plant; *GOT* ginning out turn; *SL* staple length; *MIC* micronaire; *FS* fiber strength; *WC* cuticular wax content; R^2 SSmodel/SStotal; *CoV* Coefficient of Variation

Table 2. Comparison of performance of the advance lines with the commercial check.

Variety	Year	BW		SCY	
		BW (g)	Increase to Commercial Check, FH-Lalazar (%)	SCY (g)	Increase to Commercial Check, FH-Lalazar (%)
SI-1	2021	4.65	70	94.19	61
	2022	2.27	42	128.69	87
SI-2	2021	3.51	60	75.3	51
	2022	2.28	42	95.04	82
FH-Lalazar	2021	1.39	-	36.79	-
	2022	1.32	-	17.29	-

BP number of bolls per plant; *BW* boll weight; and *SCY* seed cotton yield per plant.

Table 3. Correlation coefficients for the studied traits of advance lines, SI-1 and SI-2, during 2021 and 2022.

Trait	BW (g)	SCY (g)	WC (µg/cm2)
BW (g)	1		
SCY (g)	1* 0.96ns	1	
WC (µg/cm2)	1* 0.79ns	0.999* 0.94ns	1

BW boll weight; *SCY* seed cotton yield per plant; *WC* cuticular wax content

SUPPLEMENTARY MATERIAL

Table S1. List of cotton germplasm collected from exotic and indigenous sources for this study.

Sr. No.	Genotype	Sr. No.	Genotype	Sr. No.	Genotype	Sr. No.	Genotype	Sr. No.	Genotype
1	NIAB-878	9	FH-412	17	VH-Gulzar	25	FH-490	33	Zakariya-1
2	NIAB-1011/48	10	MNH-1016	18	Cyto-179	26	FH-Lalazar	34	Suncrop-4
3	RH-668	11	CIM-622	19	MAC-07	27	FH-142	35	Weal-AG-1606
4	BAHAR-07	12	BS-15	20	MNH-886	28	FH-152	36	Tahafuz-5
5	FH-444	13	RH-662	21	VH-189	29	Sitara-16	37	Thakkar-808
6	NIAB-545	14	IUB-65	22	IR-NIBGE-6	30	Sitara-15	38	Tarzan-5
7	NIAB-898	15	RH-Manthar	23	FH-Noor	31	FH-425	39	FH-114
8	MNH-992	16	UZ-1	24	UZ-2	32	MAC-07	40	CIM-663

Table S2. Criteria used for selection of desirable parents for crossing and progenies in the successive generations.

S. No.	Trait/Attribute Name	Trait/Attribute Threshold or Form
1.	Cuticular wax content	> 10 µg/cm^2 leaf area
2.	Yield	>10% compared to commercial check
3.	Boll weight	3-4.5 g/boll
4.	Fiber quality	Existing fiber standards
5.	Stature	Short to medium
6.	*KCS6* gene and already validated SSR markers	Presence of desirable bands of *KCS6* gene; and SSR markers NAU2083, NAU2540, and NAU3901

Table S3. List of parents selected for making crosses to develop climate smart cotton advance lines.

Sr. No	Parents
1	FH-LALAZAR
2	SIKANDAR
3	FH-425
4	FH-490
5	FH-412
6	MNH-1020
7	NIAB-1011/48
8	UZ-1
9	UZ-2
10	CIM-663

Table S4. List of crosses attempted during 2019.

Sr. No	CROSSES
1	NIAB-1011/48 x FH-425
2	UZ-1 x FH-425
3	UZ-2 x FH-425
4	FH-412 x FH-490
5	FH-Lalazar x CIM-663

6	UZ-1 x FH-412
7	UZ-2 x FH-412
8	ICI-2121 x FH-425
9	SIKANDAR-1 x FH-425
10	FH-490 x FH-425

Table S5. Mean data of parents selected for crossing.

Parents	CLCuV % age	Stature/ Plant Height (cm)	Node/ Pl	Squares/ Pl	Flowers /Pl	Bolls/Pl	Leaves size	Bolls Weight (g)	Seed Cotton Yield (g)	G. O.T (%)	Staple Length (mm)	Mike (µg/inch)	Fiber Strength (g/tex)	Cuticular wax content
FH-LALAZAR	3	150	37.2	70	48	44	Medium	4.8	211.2	38.9	28.0	4.8	35.5	64.06
SIKANDAR	9	95	32.2	40	35	33	//	2.75	90.75	41.5	28.9	4.7	29.2	74.22
FH-425	7	134	39.6	50	42	39	//	3.15	122.85	39.5	28.1	4.8	32.2	49.41
FH-490	7	160	41.1	60	45	42	//	3.25	136.5	39.0	29.1	4.6	33.0	28.73
FH-412	5	130	37.2	58	43	39	//	3.50	136.5	38.5	28.5	4.7	35.0	20.86
ICI-2121	9	125	41.1	45	38	35	//	3.35	117.25	39.1	29.8	4.5	33.5	58.87
MNH-1020	5	143	42.6	65	49	45	//	3.2	144	41.1	29.7	4.2	33.1	32.51
NIAB-1011/48	7	115	38.3	58	46	42	//	2.8	117.6	42.3	30.2	4.4	34.2	74.83
UZ-1	15	121	40.2	35	30	28	//	2.5	70	39.2	28.1	4.8	31.1	39.62
UZ-2	17	114	36.2	45	32	32	//	2.7	86.4	39	29	4.6	32.3	33.53
CIM-663	9	117	37.1	70	55	50	//	3.2	160	38.5	29.3	4.5	33.1	92.37

Table S6. Descriptive statistics for the studied traits in F_1-F_6.

Trait	Generation	Mean	Sta. dev	Minimum	Maximum
D1 (%)	F1	0.6701	0.3490	0.3	1.7333
	F2	0.3644	0.3470	0	1.3667
	F3	0.2392	0.2511	0	0.7
	F4	0.3865	0.2835	0	0.775
	F5	0.4587	0.2364	0	0.7667
	F6	0.4414	0.2610	0	0.875
SB	F1	20.7333	2.8880	15	25
	F2	21.3667	3.1237	15	27
	F3	23.5499	1.3432	21.6667	26
	F4	25.0055	1.7123	21.6667	27.3333

	F5	23.6222	1.4940	20	25.6667
	F6	25.3389	2.1050	21.5	28
MB	F1	1.4061	0.3643	0.6667	2.3333
	F2	1.1589	0.6463	0.3333	2.6667
	F3	0.4922	0.2892	0	1
	F4	1.0662	0.6040	0	2
	F5	0.9833	0.5177	0.3333	0.3333
	F6	0.7444	0.2588	0.25	1
PH (cm)	F1	104.6333	19.3079	75	140
	F2	72.2749	5.7772	63.3333	84
	F3	88.5555	7.3494	75.3333	101
	F4	78.5778	11.9484	58	99.3333
	F5	84.7944	4.4181	76	91.3333
	F6	84.9887	7.8892	71	97.33
BP	F1	49.5333	13.2163	25	78
	F2	27.8028	3.1532	21.6667	34.6667
	F3	23.6335	1.8007	21.6667	26.67
	F4	25.4722	2.7684	21.6667	29.6667
	F5	25.2611	2.3350	21	29.3333
	F6	25.9278	2.4405	21.3333	29.3333
BW (g)	F1	3.1467	0.6553	2	4.3
	F2	3.3113	0.5907	1.75	4.5
	F3	3.4133	0.5334	2.5	4.4
	F4	3.1928	0.4441	2.2667	3.625
	F5	3.1333	0.4561	2.2	3.4667
	F6	3.2505	0.4204	2.2	3.6667
SCY (g)	F1	155.5	56.9960	73.5	254.6
	F2	92.4027	20.7499	51.9167	131.15
	F3	80.4699	11.9692	55.625	96.8
	F4	82.4557	12.1945	60.375	99.7333
	F5	79.9100	16.7239	46.2	99.7333
	F6	82.1341	9.6462	63.8	100.5222
GOT (%)	F1	35.4667	1.8705	32	40
	F2	38.0490	2.1710	35.6667	45.5
	F3	37.9222	1.0775	36	40.3333
	F4	37.6	1.1578	36	39.5
	F5	37.4829	1.7049	33.66	39.75
	F6	38.0833	0.8638	36.3333	39.6667
SL (mm)	F1	25.6333	2.5928	21	30
	F2	26.6517	1.5630	21.45	28.675
	F3	27.9789	0.7453	26.5	28.925
	F4	27.6511	1.0580	25.8333	29.7667
	F5	27.2589	1.2255	25.5	28.9333
	F6	27.7350	0.9315	25.3	28.9167
MIC (µg/in)	F1	4.41	0.6535	3	5.5
	F2	4.2372	0.5215	2.8	4.75
	F3	4.3079	0.4161	3.46	4.7

	F4	4.1928	0.5274	3.1333	5
	F5	4.4094	0.3570	3.5	4.7333
	F6	4.3739	0.4608	3.3667	4.7333
FS (g/tex)	F1	27.4	2.8599	23	32
	F2	27.0163	1.0471	25.15	29.6
	F3	26.9261	0.4243	26	27.6333
	F4	26.6367	1.1414	24.7333	28.2333
	F5	28.0111	2.4172	23	32
	F6	28.6333	1.5365	26.3333	31
WC(µg/cm^2)	F1	52.7894	7.4222	31.5	61.6667
	F2	53.5844	3.6367	45.7333	58.975
	F3	52.4908	3.7963	43.8333	57.7
	F4	60.8594	5.3383	50.4333	66.7
	F5	61.4519	2.9150	56.5	65.87
	F6	60.9713	6.3400	47.5	70

DI Cotton leaf curl virus disease index; *SB* number of sympodial branches per plant; *MB* number of monopodial branches per plant; *PH* plant height: *BP* number of bolls per plant; *BW* boll weight; *SCY* seed cotton yield per plant; *GOT* ginning out turn; *SL* staple length; *MIC* micronaire; *FS* fiber strength; *WC* cuticular wax content; *Sta. dev* standard deviation

Table S7. Mean values of studied traits of cotton advance lines and the commercial check during 2021 and 2022.

Variety	Year	BW (g)	SCY (g)	WC (µg/cm2)
SI-1	2021	4.65	94.19	115.17
	2022	2.27	128.69	248.98
SI-2	2021	3.51	75.3	93.85
	2022	2.28	95.04	130.77
FH-Lalazar	2021	1.39	36.79	57.24
	2022	1.32	17.29	57.27

BW boll weight; *SCY* seed cotton yield per plant; *WC* cuticular wax content

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