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SHORT RESEARCH ARTICLE

# Weighted average absolute scores of BLUPs (WAASB) based selection of stable Asiatic cotton genotypes for the salt affected *Vertisols* of India

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## Abstract

Cotton is a major cash crop classified as moderately tolerant to salt stress (threshold ECe = 7.7 dS $m^{-1}$). In the present study, a set of 21 homozygous advanced breeding lines of cotton (*Gossypium herbaceum* L.), including one salt tolerant variety G Cot 23 was evaluated over three years to identify stable high yielding genotypes under salt stress. Weighted Average Absolute Scores of BLUPs (WAASB) stability index, which is based on single value decomposition of BLUP, was employed for this purpose. Among the 21 genotypes, CSC-025 and CSC-057 showed the highest boll weight (59.67 and 57.33 g/20 boll), seed cotton yield (1818 and 1570 Kg $ha^{-1}$) and leaf K/Na ratio (9.6 and 5.6) over the check variety G Cot 23. Considering both WAASB stability index and mean trait values, CSC-025, CSC-057 and G Cot 23 were found promising for all traits and can be considered for deployment in salt-affected *Vertisols* of India.

**Keywords:** BLUP, G × E interaction, ionic stress, salinity, yield

Soil salinity is one among the major abiotic stresses limiting seed germination, crop growth, productivity and quality (Munns and Tester 2008). Globally, it is predicted that there is a shrinkage of the cultivable area by 1–2% every year, especially in the arid and semiarid regions due to soil salinity (Kafi and Khan 2008). Presently, saline soil is spread across 100 nations, covering an area of 932.2 m ha (Rengasamy 2006). In India, 6.74 m ha area is subjected to soil salinity and is predicted to increase up to 16.2 m ha by 2050 (ICAR-CSSRI 2015).

Cotton is an important cash crop globally, and it is increasingly grown on saline agro ecosystems (Dong et al. 2020). Gradually, but surely, cotton cultivation is being pushed to less productive areas that are prone to various abiotic stresses like salinity, alkalinity, drought and flood since the productive agriculture lands are primarily diverted for food grain production. It is a moderately salt tolerant crop with a threshold salinity level of 7.7 dS $m^{-1}$ (Peng et al. 2016). Asiatic diploid cotton, *Gossypium herbaceum* L., and *Gossypium arboreum* L. are preferred for cultivation in highly saline, arid and semiarid regions of India, as compared to *Gossypium hirsutum* and Bt cotton. However, a considerable decline in the productivity and quality of fiber is observed with increasing soil salinization (Yuan et al. 2019).

Specific trait-based indirect selection for salinity tolerance requires repetitive field experiments in naturally saline soils, coupled with appropriate statistical methods to evaluate the performance and stability of genotypes (Shahzad et al. 2019). Among various stability analysis models, the AMMI model is useful in identifying the

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covariates of genotypes and environments via regression analysis using the G and E scores derived from the principal component analysis (PCA) of the GEI matrix (Gauch 2013). However, AMMI is a fixed effect model which fails to accommodate a linear mixed-effect model (LMM) structure. Best Linear Unbiased Prediction (BLUP) allows a mixed model approach which initially estimates the effects of the ANOVA model and then attributes weights (repeatability) to these effects and shrinks them towards their zero means (Piepho et al. 2008). The strength of both of these techniques was subsumed into a new quantitative genotypic stability measure called WAASB (Weighted Average Absolute Scores of BLUPs),which involves the singular value decomposition of BLUP matrix to analyze the GEI effects generated by an LMM (Olivoto et al. 2019a). WAASB × trait mean (Y) biplot is used to jointly interpret stability and trait productivity, thus exploiting broad adaptations. Although GEI has been extensively investigated by researchers for different traits in cotton, little is known about GEI under salinity stress. In the present study, we evaluated 20 Asiatic cotton genotypes and one released variety under salt affected field conditions for three consecutive years. The primary objective of this study was to identify salt tolerant, high yielding genotypes which are consistent across seasons so that they can be pushed to cotton varietal release pipeline.

### *Field experimentation and determination of ion constituents*

The material comprised of 20 near homozygous Asiatic cotton advanced breeding lines , namely, CSC-043, CSC-047, CSC-049, CSC-053, CSC-057, CSC-061, CSC-065, CSC-067, CSC-069, CSC-001, CSC-005, CSC-009, CSC-013, CSC-017, CSC-021, CSC-025, CSC-029, CSC-033, CSC-037, CSC-039 and released variety, G Cot 23 developed at ICAR-CSSRI, Regional Research Station, Bharuch, Gujarat. The experiment was conducted on salt-affected lack soil (Typic Haplustert) of the Institute Research Farm, located at Samni village (21° 52′ N, 72° 55′E) for three consecutive years during *kharif* of 2017, 2018 and 2019 in randomized complete block design with three replications. Sowing was done on July 1st week, with the onset of monsoon in all three years. The physico-chemical characteristics and various properties of the soil such as, ESP (2.39-8.02 %.), Sand (24.6-27.6 %), Silt (18.3-28.3 %), Clay (45.2-56.1 %), Organic carbon (0.43 %), Available N (160-190 kg ha$^{-1}$), Olsen P (11-26 kg ha$^{-1}$), and available K (682-840 kg ha$^{-1}$) were recorded at the experimental site during three growing seasons. The soil texture is clayey loam with pH ranging from 7.01 to 8.53 and ECe (dS m$^{-1}$) from 6.13 to 9.58.

At maturity, fully opened bolls were picked from tagged plants of each genotype and ginned using a 10-saw laboratory gin. The seed cotton and lint were weighed to calculate the boll weight (g 20 boll$^{-1}$). The seed cotton was harvested manually, and the probable environmental damage was reduced by increasing the number of pickings to four in each season. After drying, the seed cotton was weighed and expressed as seed cotton yield (SCY) kg ha$^{-1}$. Youngest fully expanded main stem leaf, which is subtending to the cotton boll was selected for ion content estimation. Three replicates from each biological replicate (total 9 samples) were sampled from each genotype to determine Na$^+$ and K$^+$ content. Ionic estimation was done using inductively coupled plasma emission spectroscopy (ICPE-9000, Shimadzu Europa GmbH, Duisburg, Germany).

The mean values of each genotype from each replication were used for statistical analysis. Each season was considered a different environment since climatic variables differed across seasons (Supplementary Fig. 1.). A preliminary individual (within each year) and pooled ANOVA were done to test the significance of different variance components. The combined ANOVA model executed is as follows:

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + \varepsilon_{ijk}$$

Where, $Y_{ijk}$ is the trait value of the $i^{th}$ genotype in $k^{th}$ year; μ is the grand mean of the experiments; $G_i$ is the $i^{th}$ genotype effect, which was considered random; $E_j$ is the $j^{th}$ environment effect assumed as fixed; $GE_{ij}$ is the interaction random effect between $i^{th}$ genotype and $j^{th}$ environment; $\varepsilon_{ijk}$ is the unaccounted residue. Genetic components of variance and heritability were extracted from this model. WAASB was calculated to assess the stability of the test genotypes. Biplots between WAASB and mean trait values were utilized to identify stable genotypes with superior performance. All the analyses were done by executing 'metan' package on R Studio statistical software Version 4.0.2 (Olivoto et al. 2020).

### *Mean performance and components of variance*

An average seed cotton yield (SCY) of 1275.5 Kg ha$^{-1}$, 20 boll weight (BW) of 48.76 g and leaf potassium to sodium ratio (K/Na) of 4.51 was recorded on salt affected black soils from the cotton genotypes evaluated across three years (2017, 2018 and 2019). Ten genotypes showed above-average SCY, the highest yielder being CSC-025 with an average yield of 1818 Kg ha$^{-1}$ across three years (Supplementary Table S1). Similarly, nine genotypes showed above-average BW and leaf K/Na ratio and CSC-025 recorded the highest 20 BW and leaf K/Na ratio (59.67 g and 9.63, respectively), across three years. The check variety, GCot23 retained its position among top 10 genotypes for all three traits. Three genotypes, CSC-025, CSC-057 and CSC-021 outperformed check variety for SCY, CSC-025, CSC-057, CSC-005 and CSC-001 showed relatively higher leaf K/Na ratio over check and CSC-025, CSC-057 and CSC-013 displayed higher BW than check variety. A significant association between SCY and BW (0.76); SCY and K/Na (0.57) and BW and K/Na (0.44) was observed. Linear regression of K/Na ratio-dependent variable SCY and BW is significant, explaining about 33% and 12% of variation, respectively. Similarly, multiple linear regressions of K/Na ratio and BW on dependent variable SCY are also significant, explaining 65% of the total variation.

A preliminary individual analysis of variance for each year showed significant differences among the genotypes in SCY, BW and K/Na ratio (Supplementary Table S2). The pooled analysis of variance indicated that the effect of environment (year), genotype and GEI components were highly significant for all the traits (Table 1). The genotypic variance ($\sigma_g^2$) was 51%, 49% and 71% for SCY, BW and leaf K/Na ratio, respectively. GEI and E components jointly contributed 49%, 51% and 29% of total variance in SCY, BW and K/Na ratio, respectively. Under saline conditions, plants take up sodium, and therefore K/Na ratio is a potential indicator of sodium or potassium uptake and an important aspect of cellular ion homeostasis (Reddy et al. 2017). Between E and GEI, the variance due to E is more prominent than that is due to GEI (Table 1). A significant role of environment and GEI for SCY and BW was also reported in previous studies conducted under unstressed conditions (Campbell and Jones 2005; Zeng et al. 2014; Farias et al. 2016)**.** Heritability (broad sense, $h^2$) was highest for leaf K/Na ratio compared to SCY and BW, indicating that selection for tolerant genotypes can safely be done through the K/Na ratio. Genotypic coefficient of variation (GCV) was almost double that of the environmental coefficient (ECV) for K/Na ratio, while it was also higher for SCY and BW (Table 1). Similar GCV in cotton (4.7 - 31.5%) with an average of 14.3% was reported by Mora et al. (2007). Contrary to previous studies (Mukoyi et al. 2015; Orawu et al. 2017; Riaz et al. 2019), we evaluated the genotypes at a single location for three consecutive years and hence the effect of the environment was less pronounced as compared to G and GEI.

**Table 1.** Pooled analysis of variance, variance component and genetic parameters for seed cotton yield, boll weight and leaf K/Na ratio of 21 cotton genotypes

| Source | DF | SCY | Boll weight | Leaf K/Na |
|---|---|---|---|---|
| **Analysis of variance** | | | | |
| Year | 2 | 1567115.07*** | 470.48*** | 52.73*** |
| Replication | 6 | 12103.11 | 23.38 | 0.80 |
| Genotype | 20 | 430375.51*** | 234.41*** | 18.55*** |
| GEI | 40 | 77467.45*** | 38.35*** | 1.28*** |
| Residuals | 120 | 16903.1 | 14.38 | 0.52 |
| **Variance components and genetic parameters** | | | | |
| $\sigma_g^2$ | | 39213 (51.39)† | 21.78(49.33) | 1.92 (71.23) |
| $\sigma_{ge}^2$ | | 20188 (26.46) | 7.98(18.09) | 0.26 (9.48) |
| $\sigma_e^2$ | | 16903 (22.15) | 14.39(32.58) | 0.52 (19.28) |
| $\sigma_p^2$ | | 76304 (100) | 44.16(100) | 2.69(100) |
| $h^2$ | | 0.5 | 0.5 | 0.7 |
| GCV | | 15.52 | 9.57 | 30.72 |
| ECV | | 10.19 | 7.78 | 15.99 |
| CV ratio | | 1.52 | 1.23 | 1.92 |
| $\sigma_{ge}^2/\sigma_g^2$ | | 0.51 | 0.37 | 0.13 |

†Figures in the parenthesis indicate percentage of total phenotypic variance

DF- degrees of freedom; SCY- Seed cotton yield; GEI- genotype × environment interaction; *** -significant at $p < .001$;*- significant at $p < 0.05$; $\sigma_g^2$, genotypic variance; $\sigma_{ge}^2$, genotype by environment variance; $\sigma_e^2$; residual variance; $\sigma_p^2$, phenotypic variance; $h^2$, broad-sense heritability; $r_{ge}$ , correlation between genotypic values across seasons; GCV, genotypic coefficient of variation; ECV, environment coefficient of variation

### *Mean vs stability for salt tolerance*

A joint interpretation of individual trait performance and stability of genotypes across the years is presented in the four quadrants of Y×WAASB biplot in Fig. 1(a-c). The genotypes or environments (years/season) placed in quadrant I are unstable or environments with high discrimination ability and low productivity below the grand mean. In quadrant

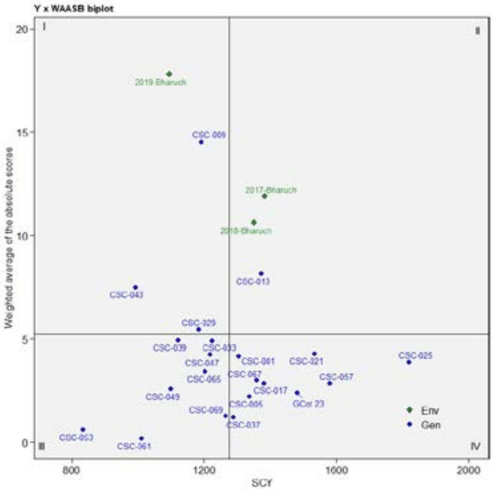

**Fig. 1a.** Biplots for seed cotton yield vs. weighted average absolute scores for the best linear unbiased predictions of 21 cotton genotypes evaluated across the years

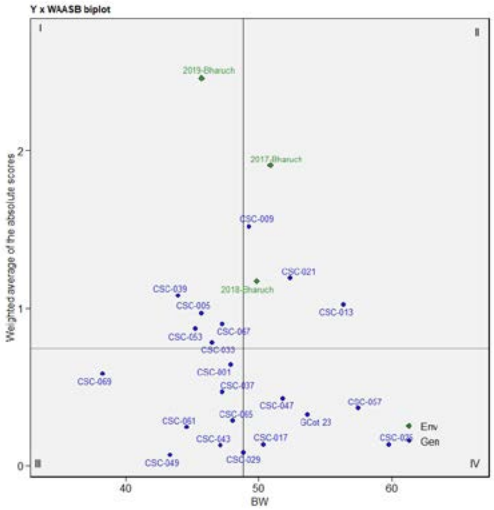

**Fig. 1b.** Biplots for boll weight vs. weighted average absolute scores for the best linear unbiased predictions of 21 cotton genotypes evaluated across the years

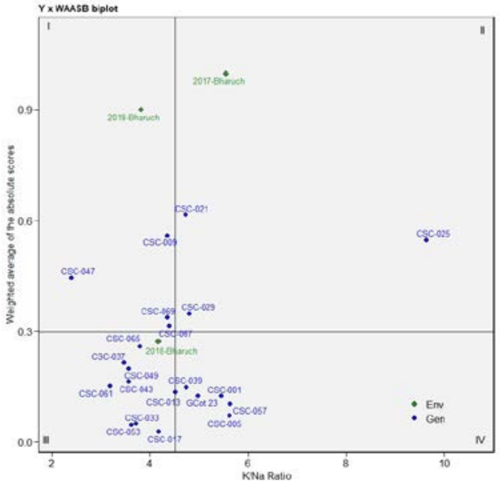

**Fig. 1c:** Biplots for leaf K/Na ratio vs. weighted average absolute scores for the best linear unbiased predictions of 21 cotton genotypes evaluated across the years

II, the productivity of the genotype is above the grand mean but unstable. The environments in quadrant II were good discriminating environments with high magnitudes of the response variable. Genotypes in quadrant III have low productivity but stable due to the lower values of WAASB. The environments in quadrant III is considered poorly productive and with low discrimination ability. The genotypes in quadrant IV are highly productive and broadly adapted due to the high magnitude of the response variable and high stability performance. The present study includes the years 2017 and 2018 in quadrant II, followed by 2019 in quadrant I of the WAASB biplot for SCY and BW. However, for leaf K/Na ratio, years 2017, 2018 and 2019 are included in quadrant II III and I, respectively. This indicates that all three seasons were good discriminating environments for SCY and boll weight, whereas the season 2018 is undiscriminating for leaf K/Na ratio. The season 2019 is unfavorable and less productive with the high discriminating ability for all three traits and is placed in quadrant I. Out of the 21 genotypes, three for SCY, five for BW and four for K/Na ratio are included in quadrant I.

CSC-067 was the common genotype among the nine genotypes placed in quadrant I for BW and leaf K/Na. Similarly, CSC-009 remained common among the seven genotypes included in quadrant I for SCY and K/Na ratio (Fig. 1a-c.). These genotypes were unstable and poor yielders. CSC-013 was the sole genotype included in quadrant II for SCY, indicating its high mean but variable performance. CSC-009, CSC-021, and CSC-013 for BW and CSC-021, CSC-025 and CSC-029 for K/Na ratio are also included in the second quadrant. These genotypes displayed superior performance in either of the years, but not all. Majority of the genotypes are included in quadrant III and IV of WAASB biplot. These genotypes are highly stable, but at the cost of their poor performance. Similarly, nine genotypes for SCY, six genotypes for BW and K/Na ratio are included in quadrant IV. This group is more relevant since these genotypes had consistently superior trait values across seasons. Different genotypes are found in the fourth quadrant for different traits, with a few being common between two or more traits. CSC-017 and CSC-025 were found common in quadrant IV for SCY and BW. Between these two genotypes, CSC-025 registered lower WAASB and highest trait values for both SCY and BW. However, for leaf K/Na ratio it is included in quadrant II, which needs to be evaluated further. GCot23 and CSC-057 are also retained in quadrant IV for all the traits (Fig.1a-c.). Altogether, CSC-025, CSC-057 and G Cot 23 were selected as the most stable, high-performing genotypes for salt affected *Vertisols*.

## Authors' contribution

Conceptualization of research (TV, IP, ARC); Designing of the experiments (TV, IP); Contribution of experimental materials (IP); Execution of field/lab experiments and data collection (TV, BL, IP, SK); Analysis of data and interpretation (TV, BL, RK); Preparation of the manuscript (TV, BL, RK, ARC, PCS).

## Supplementary materials

Supplementary Tables S1 and S2 and a Supplementary Fig. 1 are presented.

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**Supplementary Table S1.** Mean values of seed cotton yield, 20 boll weight and leaf K/Na ratio of 21 genotypes across three years of study

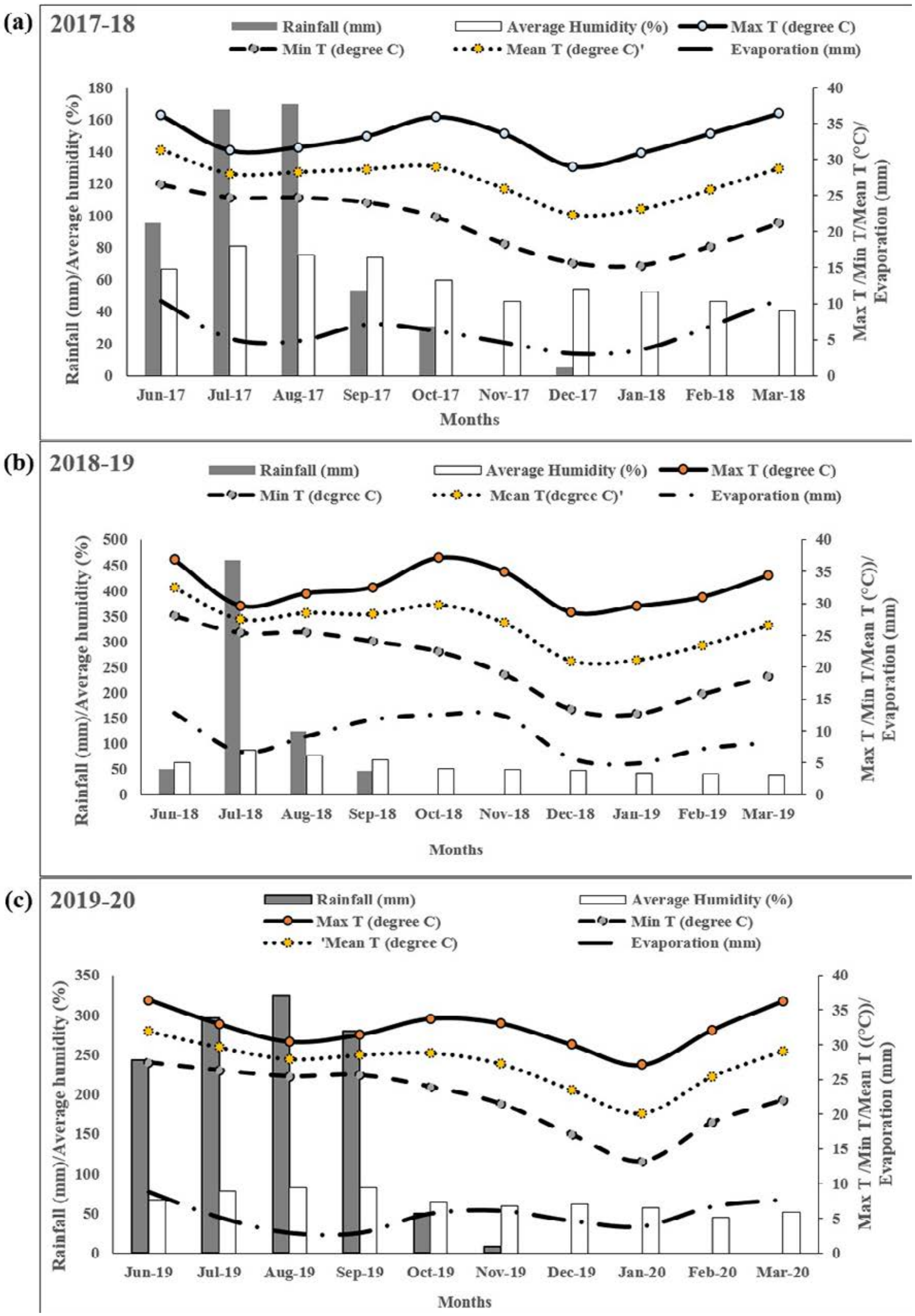
| Genotype | SCY (Kg ha$^{-1}$) | | | | Boll weight (g) | | | | K/Na ratio | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2017 | 2018 | 2019 | mean | 2017 | 2018 | 2019 | mean | 2017 | 2018 | 2019 | mean |
| CSC-043 | 942.00 | 973.00 | 1058.00 | 991.00 | 49.00 | 48.00 | 45.00 | 47.33 | 4.30 | 4.00 | 2.40 | 3.57 |
| CSC-047 | 1251.00 | 1218.00 | 1182.00 | 1217.00 | 53.00 | 52.00 | 51.00 | 52.00 | 2.50 | 2.20 | 2.50 | 2.40 |
| CSC-049 | 1258.00 | 1208.00 | 830.00 | 1098.67 | 45.00 | 45.00 | 40.00 | 43.33 | 4.20 | 3.30 | 3.20 | 3.57 |
| CSC-053 | 936.00 | 924.00 | 640.00 | 833.33 | 50.00 | 48.00 | 37.00 | 45.00 | 4.80 | 3.20 | 2.90 | 3.63 |
| CSC-057 | 1717.00 | 1720.00 | 1302.00 | 1579.67 | 61.00 | 58.00 | 53.00 | 57.33 | 6.90 | 5.10 | 4.80 | 5.60 |
| CSC-061 | 1113.00 | 1085.00 | 833.00 | 1010.33 | 48.00 | 45.00 | 40.00 | 44.33 | 4.00 | 2.70 | 2.80 | 3.17 |
| CSC-065 | 1200.00 | 1292.00 | 1114.00 | 1202.00 | 48.00 | 52.00 | 44.00 | 48.00 | 5.40 | 3.40 | 2.60 | 3.80 |
| CSC-067 | 1481.00 | 1337.00 | 1259.00 | 1359.00 | 47.00 | 46.00 | 49.00 | 47.33 | 6.10 | 4.00 | 3.10 | 4.40 |
| CSC-069 | 1440.00 | 1289.00 | 1064.00 | 1264.33 | 39.00 | 37.00 | 38.00 | 38.00 | 6.30 | 3.40 | 3.40 | 4.37 |
| CSC-001 | 1201.00 | 1518.00 | 1191.00 | 1303.33 | 46.00 | 50.00 | 47.00 | 47.67 | 6.60 | 4.60 | 5.10 | 5.43 |
| CSC-005 | 1360.00 | 1534.00 | 1116.00 | 1336.67 | 43.00 | 48.00 | 47.00 | 46.00 | 6.70 | 5.10 | 5.10 | 5.63 |
| CSC-009 | 1515.00 | 1538.00 | 520.00 | 1191.00 | 54.00 | 55.00 | 38.00 | 49.00 | 4.20 | 4.10 | 4.70 | 4.33 |
| CSC-013 | 1653.00 | 1542.00 | 922.00 | 1372.33 | 62.00 | 59.00 | 48.00 | 56.33 | 5.40 | 4.10 | 4.10 | 4.53 |
| CSC-017 | 1439.00 | 1572.00 | 1129.00 | 1380.00 | 51.00 | 53.00 | 47.00 | 50.33 | 5.10 | 3.90 | 3.50 | 4.17 |
| CSC-021 | 1528.00 | 1589.00 | 1484.00 | 1533.67 | 51.00 | 50.00 | 56.00 | 52.33 | 6.80 | 4.80 | 2.60 | 4.73 |
| CSC-025 | 1892.00 | 1807.00 | 1755.00 | 1818.00 | 62.00 | 60.00 | 57.00 | 59.67 | 11.70 | 9.50 | 7.70 | 9.63 |
| CSC-029 | 1236.00 | 1135.00 | 1178.00 | 1183.00 | 50.00 | 50.00 | 46.00 | 48.67 | 5.30 | 4.10 | 4.90 | 4.77 |
| CSC-033 | 1268.00 | 1199.00 | 1207.00 | 1224.67 | 46.00 | 46.00 | 48.00 | 46.67 | 4.70 | 3.30 | 3.10 | 3.70 |
| CSC-037 | 1470.00 | 1274.00 | 1118.00 | 1287.33 | 53.00 | 46.00 | 43.00 | 47.33 | 4.10 | 3.10 | 3.20 | 3.47 |
| CSC-039 | 1355.00 | 1217.00 | 787.00 | 1119.67 | 50.00 | 46.00 | 35.00 | 43.67 | 5.90 | 4.70 | 3.70 | 4.77 |
| G Cot 23 | 1756.00 | 1396.00 | 1290.00 | 1480.67 | 58.00 | 53.00 | 50.00 | 53.67 | 5.70 | 4.90 | 4.40 | 5.00 |
| Mean | 1381.48 | 1350.81 | 1094.24 | 1275.51 | 50.76 | 49.86 | 45.67 | 48.76 | 5.56 | 4.17 | 3.80 | 4.51 |

SCY- Seed cotton yield

**Supplementary Table S2.** Individual analysis of variance for SCY, BW and K/Na ratio of 21 cotton genotypes in individual seasons (2017, 2018 and 2019)

| Environment | Source | Df | Mean sum of squares | | |
|---|---|---|---|---|---|
| | | | SCY | BW | K/Na ratio |
| 2017 | Replication | 2 | 13418.63 | 36.21 | 1.55 |
| | Genotype | 20 | 186952.73** | 113.92** | 9.82** |
| | Error | 40 | 17908.58 | 15.54 | 0.80 |
| 2018 | Replication | 2 | 3554.57 | 19.83 | 0.27 |
| | Genotype | 20 | 167881.88** | 87.29** | 6.40** |
| | Error | 40 | 6386.16 | 17.94 | 0.11 |
| 2019 | Replication | 2 | 19242.03 | 14.11 | 0.69 |
| | Genotype | 20 | 230494.79** | 109.92** | 4.92** |
| | Error | 40 | 26410.08 | 9.68 | 0.65 |

SCY, Seed cotton yield; BW, Boll weight; **significant at $p < .01$



**Supplementary Fig.1a-c. Seasonal environmental variables during cotton growth period (June to March) in 2017-2020**