Selection of Genotypes and Contributing Characters to Improve Seed Cotton Yield of Upland Cotton (Gossypium hirsutum L.)

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Authors’ contributions

This work was carried out in collaboration among all authors. Author MMAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SK and MFA managed the analyses of the study. Authors BKB and MH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was conducted at Cotton Research Centre, Chowgacha, Jessore during 2015-2016 with twenty genetically diverged genotypes, with a view to select desirable genotypes and important characters enhancing seed cotton yield in upland cotton. The experiment was laid out in RCBD with three replications. The unit plot size was 4.5 m x 4.5 m and the spacing, 45 cm × 90 cm. Data were recorded from randomly selected ten plants on monopodial branches plant-1, primary sympodial branches plant-1, secondary sympodial branches plant-1, main stem nodes plant-1, days to 1st (5%) flowering, days to 1st boll splitting, bolls plant-1, single boll weight (g), unburst bolls plant-1, plant height (cm), seeds boll-1, seed cotton yield (kg ha-1). The genotype JA-08/D produced the highest seed cotton yield (3430 kg ha-1) followed by two genotypes JA-08/C (3329 kg ha-1) and JA-08/E (3226 kg ha-1). Though high heritability (90.36-97.46%) were estimated for four characters but high heritability along with high genetic advance values were observed for bolls plant-1, boll...
weight and seeds boll−1. The moderate heritability (83.58%) with high genetic advance (48.54%) suggests seed cotton yield is controlled by additive and non-additive genetic factors. In general, genotypic correlation coefficient was higher than corresponding phenotypic correlation coefficient due to inherent potential of the genotypes for expressing the individual characters. However, six characters, secondary sympodial branches plant−1, main stem nodes plant−1, days to 1st flowering, bolls plant−1, boll weight and seeds boll−1 showed positive and significant association. Upon partitioning the correlation coefficients of seed cotton yield with other characters confirmed significant contribution of three characters like bolls plant−1, boll weight and seeds boll−1. Therefore, three genotypes, JA-08/D, JA-08/C and JA-08/E and three characters, bolls plant−1, boll weight and seeds boll−1 may be considered by the breeders for improving seed cotton yield in upland cotton.

**Keywords:** Heritability; genetic advance; correlation; path analysis; upland cotton.

1. INTRODUCTION

Cotton is one of the most important fiber crops popularly known as the "White Gold". It belongs to genus Gossypium under tribe Gossypieae, family Malvaceae comprises 50 species. Out of these, four species are commercially cultivated like, G. herbaceum (2n = 26), G. arboreum (2n = 26), G. hirsutum (2n = 52) and G. barbadense (2n = 52). Cotton is a fiber, oil and protein yielding crop [1] but it had evolved as a fiber crop; the upland Cotton (Gossypium hirsutum L.) is the most important cash and fiber yielding crop [2,3]. The species is called upland cotton, grown in 90% of the cotton area while the Egyptian cotton (G. barbadense) is grown only in 9% area [4].

Always the cotton breeders are continuously applying different breeding methods through exploiting diversified genetic resources of cotton to develop high fiber yielding varieties [5,6] but before commencing any breeding program for improvement of seed cotton yield, it is essential to understand different yield enhancing components of cotton [7,8]. Therefore, cotton breeders must have sufficient knowledge on genetic parameters such as coefficient of variation, heritability, genetic advance, and correlation among different pairs of characters and partition of correlation confident of seed cotton yield with contributing characters [6,9,10]. It is noted that heritability when conjugated with genetic advance becomes high, breeders may utilize this information as a powerful tool to select appropriate breeding program [6,11,12] because researchers reported different magnitudes of heritability along with genetic advance for seed cotton yield and contributing characters [6,13,14,15,16,17]. Therefore, there is a scope of study to ascertain heritability associated with genetic advance for seed cotton yield contributing characters of upland cotton.

Since, seed cotton yield and other economic characters in cotton are the complex quantitative characters, direct selection might mislead the breeding program, as the characters are influenced by environmental factors. In such situation, plant breeders determine correlation coefficient between pairs of characters in all possible combinations to pave the way of understanding the associations prevailing between seed cotton yield and contributing characters [18], which eventually help to assess genetic makeup of a genotype as a whole [19]. Thus, study on correlation coefficient provides a way to predict how variation does prevail in a genotype to harmonize the variation observed for other characters [20,21]. So, it is important for the breeders to know all possible associations among the characters while applying a breeding method for selecting characters. Furthermore, path coefficient analysis, proposed by [22,23] permits a better understanding of character association through separation of correlation coefficients into direct and indirect effects on the characters of interest [23,24]. In cotton such type studies have done by several researchers, suggested for the improvement seed cotton yield potential [23,25,26,27]. Keeping in view the importance of genetic studies of different quantitative characters, the present investigation was under taken to assess different genetic parameters, correlation and path analysis for seed cotton yield and yield contributing characters in diversified upland cotton genotypes.

2. MATERIALS AND METHODS

2.1 Experimental Design and Data Recording

The experiment was conducted at Cotton Research Centre, Chawgacha, Jessore, Bangladesh during 2015-2016 cropping season on twenty genetically diversified upland cotton genotypes in a RCBD with three replications. The unit plot and spacing were 4.5 m x 4.5 m and 45 cm x 90 cm respectively. Two seeds /hill were
planted on 1st July, 2015 and one seedling from each hill was uprooted just after completion of germination. All intercultural operations were properly carried out during entire crop growth. Data were recorded from ten randomly selected plants on MB/P = Monopodial (vegetative) branches plant⁻¹, PSB/P = Primary sympodial branches plant⁻¹, SSB/P = Secondary sympodial branches plant⁻¹, MSN/P = Main stem node plant⁻¹, DFF = Days to 1st flowering (5%), DFBS = Days to 1st boll splitting (5%), B/P = Bolls plant⁻¹, SBW = Single weight (g), UBB/P = Unburst bolls plant⁻¹, PH = Plant height (cm), S/B = Seeds boll⁻¹, and SCY = Seed cotton yield (kg ha⁻¹).

2.2 Statistical Analysis of the Recorded Data

Analysis of variance (ANOVA) of the observations recorded on different characters was carried out as per the standard procedure suggested by [28]. According to [29], genotypic and phenotypic coefficients of variation were estimated based on the genotypic and phenotypic variances. The genotypic and phenotypic coefficients of variation were categorized as per the method suggested by [30]. Heritability in broad sense and genetic advance were calculated as suggested by [31]. Genotypic and phenotypic correlation coefficients were estimated though the statistical technique applied by [32]. By using the method of [33, 34], significance of genotypic and phenotypic correlation coefficients was tested. Path coefficient analysis was performed using the method suggested by [35].

3. RESULTS AND DISCUSSION

3.1 Mean Performance of the Upland Cotton Genotypes

The means of twelve characters are presented in Table 1. The coefficient of variation against each character was reasonable, ranged from 6.82 - 18.94% suggests good precision of experiment and negligible sampling error during data recording. The values of each character against twenty genotypes are tested by LSD (least significant difference) at 5% level of probability. All the characters significantly varied among the genotypes. The highest bolls plant⁻¹ (37.9) was recorded from BC-05, followed by the genotypes, CB-10 and JA-08/A with 33.4 and 33.5 bolls plant⁻¹ respectively. The genotype, JA-054 produced the lowest bolls plant⁻¹ (24.9). The range of single boll weight ranged from 4.17 - 6.17 g and the highest single boll weight was recorded from JA-08/C, followed by JA-054 with 5.80 g and CB-9 with 5.97 g. The highest seeds boll⁻¹ (35.0) was recorded from JA-0526, followed by JA-08/C and JA-08/D with 29.9 and 28.2 respectively. The genotypes, CB-9 and CB-11 both produced the lowest seeds boll⁻¹ (23.3). The resultant product seed cotton yield greatly varied among the twenty genotypes; the highest seed cotton yield was obtained from JA-08/D with 3430 kg ha⁻¹, followed by JA-08/C and JA-08/E with mean seed cotton yield, 3329 and 3226 kg ha⁻¹ respectively. Therefore, the seed cotton yield potential of these three genotypes were unparalleled to other lower seed cotton yielding genotypes.

3.2 Genetic Parameters Seed Cotton Yield and Other Eleven Characters of Upland Cotton

Adequate variability was present for seed cotton yield and yield contributing characters in the cotton genotypes. In a crop breeding improvement is possible when extent of genetic variability becomes wide in the population [36]. The different genetic parameters for twelve characters including seed cotton yield are presented in Table 2. A wide range of difference between PCV (phenotypic coefficient of variation) and GCV (genotypic coefficient of variation) were measured in days to 1st boll splitting (PCV: GCV=80.45:65.94), plant height (PCV: GCV=72.45:60.920) and seed cotton yield (PCV: GCV=330.12: 205.43). The results suggest role of environment upon expression of the characters. The minimum difference between PCV and GCV for other characters indicate less influence of environment, hence scope of improvement through direct selection would not be effective. The important characters, bolls plant⁻¹, single boll weight and seeds boll⁻¹ exhibited high heritability, therefore, reliable selection could be operated expecting progress in next generation. Besides, high heritability of a character indicates additive action of alleles controlling the character. The character, seed cotton yield had 83.58% heritability, which is moderate as compared to other characters but the character showed remarkable genetic advance, 48.54%, suggests both additive and non-additive genetic factors are involved to express the characters [37, 38, 39, 40, 41, 42, 43]. The character, plant height although had high heritability (97.46%) but its genetic advance as percentage of mean was low, 9.42%, suggests the character is controlled both additive and non-additive with a complex interaction of genetic factors.
Table 1. Means seed cotton yield and its related characters in upland cotton genotypes

| Genotypes | MB/P | PSB/P | SSB/P | MSN/P | DFF | DFBS | B/P | SBW (g) | UBB/P | PH (cm) | S/B | SCY (kg ha⁻¹) |
|-----------|------|-------|-------|-------|-----|------|-----|---------|-------|---------|-----|----------------|
| JA-08/A   | 1.93 | 14.47 | 11.20 | 6.23  | 53  | 103  | 33.5| 4.74    | 1.4   | 106.37  | 27.9| 3012          |
| JA-08/B   | 1.60 | 13.43 | 9.53  | 6.00  | 50  | 105  | 26.7| 5.03    | 1.8   | 96.60   | 24.6| 2641          |
| JA-08/C   | 1.87 | 14.67 | 8.13  | 6.23  | 51  | 106  | 31.5| 6.17    | 1.4   | 101.73  | 29.9| 3329          |
| JA-08/D   | 4.27 | 26.00 | 21.33 | 7.13  | 51  | 109  | 33.2| 4.93    | 1.4   | 108.73  | 28.2| 3430          |
| JA-08/E   | 1.80 | 15.40 | 8.07  | 6.40  | 53  | 107  | 31.0| 5.51    | 1.6   | 94.33   | 28.1| 3226          |
| JA-0541   | 1.47 | 17.37 | 9.50  | 6.07  | 49  | 108  | 28.2| 5.46    | 1.5   | 110.07  | 25.5| 2711          |
| JA-08/9   | 1.40 | 17.23 | 6.53  | 5.27  | 48  | 102  | 31.7| 4.17    | 2.3   | 91.80   | 26.8| 2904          |
| JA-054    | 1.53 | 14.93 | 9.03  | 5.73  | 51  | 105  | 24.9| 5.80    | 1.6   | 101.33  | 26.0| 2810          |
| JA-0526   | 2.33 | 19.07 | 13.53 | 6.23  | 52  | 104  | 30.0| 5.27    | 1.6   | 108.33  | 35.0| 2703          |
| JA-0510   | 1.97 | 15.10 | 9.00  | 5.93  | 50  | 104  | 29.0| 4.95    | 1.5   | 102.13  | 26.3| 2799          |
| BC-088    | 2.30 | 21.97 | 13.50 | 6.33  | 50  | 106  | 29.0| 4.75    | 1.0   | 116.47  | 22.9| 2580          |
| BC-0303   | 2.57 | 20.17 | 12.17 | 6.40  | 53  | 109  | 33.0| 4.49    | 1.2   | 130.93  | 27.4| 3089          |
| BC-0406   | 3.20 | 22.50 | 16.23 | 6.53  | 51  | 107  | 37.9| 4.47    | 1.8   | 118.73  | 25.6| 2969          |
| BC-051    | 2.03 | 16.60 | 13.23 | 6.33  | 47  | 104  | 34.5| 4.33    | 1.6   | 130.00  | 27.1| 2973          |
| BC-0342   | 2.77 | 20.10 | 16.57 | 7.03  | 51  | 106  | 29.9| 5.40    | 1.2   | 131.27  | 26.5| 2758          |
| BC-037    | 2.67 | 21.87 | 13.47 | 6.40  | 56  | 109  | 32.4| 4.99    | 1.9   | 128.80  | 23.0| 2466          |
| BC-0188   | 2.13 | 22.17 | 15.13 | 6.73  | 53  | 107  | 32.7| 4.36    | 1.5   | 131.27  | 26.5| 2758          |
| CB-9      | 3.17 | 21.20 | 14.73 | 7.73  | 56  | 115  | 25.6| 5.97    | 1.5   | 104.70  | 23.3| 2389          |
| CB-10     | 2.63 | 23.33 | 15.50 | 6.57  | 50  | 116  | 33.4| 5.07    | 1.7   | 118.67  | 27.0| 3205          |
| CB-11     | 0.67 | 16.13 | 6.63  | 6.27  | 50  | 105  | 26.9| 5.34    | 1.5   | 119.73  | 23.3| 2483          |
| CV (%)    | 12.36| 14.70 | 9.56  | 6.82  | 7.17| 11.24| 9.98| 7.53    | 15.67  | 15.69   | 8.22| 18.94         |
| LSD       | 0.97 | 5.27  | 3.16  | 0.85  | 0.98| 0.65 | 4.83| 0.64    | 0.47   | 10.22   | 2.05| 504.68        |

Here, MB/P = Monopodial branches plant⁻¹, PSB/P = Primary sympodial branches plant⁻¹, SSB/P = Secondary sympodial branches plant⁻¹, MSN/P = Main stem node plant⁻¹, DFF = Days to 1st flowering (5%), DFBS = Days to 1st boll splitting (5%), B/P = Bolls plant⁻¹, SBW = Single boll weight (g), UBB/P = Unburst bolls plant⁻¹, PH = Plant height (cm), S/B = Seeds boll⁻¹ and SCY = Seed cotton yield (kg ha⁻¹)
Table 3. Genotypic ($r_g$) and phenotypic ($r_p$) correlation coefficients of twelve characters in upland cotton

| Characters | MB/P | PSB/P | SSB/P | MSN/P | DFF | DFBS | B/P | SBW (g) | UBB/P | PH(cm) | S/B | SCY (kg ha$^{-1}$) |
|------------|------|-------|-------|-------|-----|------|-----|---------|-------|--------|-----|-------------------|
| $r_g$      | 1.00 | -0.240 | 0.763** | 0.281 | -0.563* | 0.094 | -0.783** | 0.438 | -0.820** | 0.095 | 0.275 | -0.850.** |
| $r_p$      | 1.00 | -0.221 | 0.710** | 0.259 | -0.529* | 0.087 | -0.730** | 0.320 | -0.794** | 0.088 | 0.251 | -0.801.** |
| $r_g$      | 1.00 | 0.009 | 0.105 | 0.220 | -0.489 | 0.390 | 0.390 | 0.007 | 0382. | 0.117 | 0.474 |
| $r_p$      | 1.00 | -0.003 | 0.096 | 0.187 | -0.453 | 0.299 | 0.354 | 0.006 | 0.321 | 0.111 | 0.457 |
| $r_g$      | 1.00 | 0.304 | -0.132 | 0.094 | 0.190 | 0.667** | 0.109 | 0.311 | 0.680** | 0.889** |
| $r_p$      | 1.00 | 0.295 | -0.123 | 0.092 | 0.153 | 0.651** | 0.099 | 0.275 | 0.653** | 0.875** |
| $r_g$      | 1.00 | 0.774** | 0.874** | 0.136 | 0.082 | 0.472 | 0.768** | 0.099 | 0.843** |
| $r_p$      | 1.00 | 0.583* | 0.827** | 0.119 | 0.079 | 0.459 | 0.717** | 0.093 | 0.0818.** |
| $r_g$      | 1.00 | 0.651** | 0.765** | 0.212 | -0.009 | 0.348 | 0.231 | 0.0775.** |
| $r_p$      | 1.00 | 0.650** | 0.732** | 0.200 | -0.008 | 0.333 | 0.202 | 0.749** |
| $r_g$      | 1.00 | -0.107 | 0.357 | 0.273 | 0.110 | 0.200 | 0.273 |
| $r_p$      | 1.00 | -0.100 | 0.355 | 0.272 | 0.101 | 0.197 | 0.259 |
| $r_g$      | 1.00 | -0.684** | 0.795** | 0.280 | 0.239 | 0.786** |
| $r_p$      | 1.00 | -0.666.** | 0.784** | 0.276 | 0.224 | 0.779** |
| $r_g$      | 1.00 | 0.240 | 0.163 | 0.793** | 0.689** |
| $r_p$      | 1.00 | 0.236 | 0.154 | 0.768** | 0.675** |
| $r_g$      | 1.00 | 0.300 | 0.107 | -0.681** |
| $r_p$      | 1.00 | 0.289 | 0.097 | -0.664** |
Here, MB/P = Monopodial branches plant $^{-1}$, PSB/P = Primary sympodial branches plant $^{-1}$, SSB/P = Secondary sympodial branches plant $^{-1}$, MSN/P = Main stem node plant $^{-1}$, DFF = Days to 1st flowering (5%), DFBS = Days to 1st boll splitting (5%), B/P = Bolls plant $^{-1}$, SBW = Single boll weight (g), UBB/P = Unburst bolls plant $^{-1}$, PH = Plant height (cm), S/B = Seeds boll $^{-1}$, and SCY = Seed cotton yield (kg ha$^{-1}$).

**Indicates significant at 1% level of probability

Table 4. Path coefficient analysis on correlation coefficients of eleven characters with seed cotton yield

| Characters | MB/P  | PSB/P  | SSB/P  | MSN/P  | DFF   | DFBS  | B/P   | SBW   | UBB/P  | PH    | S/B   | SCY   | r with SCY |
|------------|-------|--------|--------|--------|-------|-------|-------|-------|--------|-------|-------|-------|------------|
| MB/P       | 0.754 | -0.410 | 0.280  | 0.525  | 0.209 | -0.518| -0.362| -0.127| 0.423  | 0.306 | -0.422| -0.850**|
| PSB/P       | -0.375| 0.149  | 0.097  | 0.130  | 0.102 | 0.138 | 0.321 | 0.030 | -0.220 | -0.198| 0.300 | 0.474 |
| SSB/P       | -0.643| 0.172  | **1.384**| 0.277  | -0.407| -0.768| 0.409 | -0.087| 0.557  | 0.626 | 0.503 | 0.889**|
| MSN/P       | 0.256 | 0.062  | 0.202  | **0.163**| -0.145| -0.100| 0.107 | 0.008 | 0.048  | 0.137 | 0.105 | 0.843**|
| DFF         | -0.479| 0.270  | 0.020  | -0.286 | **0.597**| 0.518 | 0.291 | 0.135 | -0.593 | 0.041 | 0.261 | 0.775**|
| DFBS        | -0.175| 0.048  | 0.105  | -0.229 | 0.200 | **0.328**| 0.261 | -0.194| -0.251 | 0.056 | 0.124 | 0.273 |
| B/P         | -0.267| 0.129  | 0.154  | 0.038  | 0.303 | -0.389| **1.526**| -0.472| -0.150 | 0.171 | -0.257| 0.786**|
| BW          | -0.365| 0.208  | 0.122  | -0.338 | 0.050 | -0.487| -0.673 | **1.568**| -0.290 | 0.243 | 0.651 | 0.689**|
| UBB/P       | 0.052 | 0.073  | -0.104 | 0.111  | -0.278| 0.168 | 0.129 | -0.205| **-0.872**| 0.106 | 0.139 | -0.681**|
| PH          | -0.189| 0.155  | 0.004  | 0.216  | -0.316| 0.107 | -0.152| 0.070 | 0.144  | **0.104**| 0.218 | 0.351 |
| S/B         | -0.156| 0.263  | 0.105  | -0.587 | 0.120 | -0.456| -0.731 | 0.198  | -0.149 | 0.104 | **1.982**| 0.693**|

The bold figures on the diagonal are the direct effects and other figures are indirect effects except correlation coefficient.

Residual effect = 0.0415
Fig. 1. Diagrammatic representation of path analysis carried on correlation of seed cotton yield
3.3 Genotypic and Phenotypic Correlation Coefficients

For fruitful gain from selection, nature of association of seed cotton yield with its accelerating characters must be ascertained, otherwise arbitrary selection would mislead the entire breeding program. For this reason, taking twelve characters in consideration all possible ways of correlation coefficients were computed among pairs of characters both at genotypic and phenotypic levels (Table 3). Therefore, correlation coefficients determination among seed cotton yield and its related components were estimated both at genotypic and phenotypic levels, where genotypic correlation coefficient was higher than corresponding phenotypic correlation coefficient and both the coefficients were in same direction suggest relative constancy of the genotypes in relation to expression of the characters under study and masking of environmental effects on genetic potential of the characters had understood (31). The resultant product, seed cotton yield showed positive and significant association with secondary sympodial branches plant\(^{-1}\), main stem node plant\(^{-1}\), days to 1\(^{st}\) flowering, bolls plant\(^{-1}\), single boll weight and seeds boll\(^{-1}\) both at genotypic and phenotypic levels (Table 3). These results confirmed the findings reported by [44,45]. For such important morphological parameters showing positive and strong significant association with seed cotton yield, rational improvement is possible through simultaneous selection for these characters prior to hybridization program (36). Two characters, monopodial branches plant\(^{-1}\) and unburst bolls plant\(^{-1}\) showed negative and significant association both at genotypic and phenotypic levels. Therefore, selection of the two characters would not be effective to increase seed cotton yield. The correlation coefficient of plant height with seed cotton yield was positive but non-significant but positive and significant correlation between two characters was reported by [46,47,48,49].

3.4 Phenotypic Path Analysis on Seed Cotton Yield and Its Contributing Characters

Since genotypic correlation coefficients are intrinsic potential of the genotypes and have more significant in developing the relationship than phenotypic correlation coefficients and phenotypic correlation coefficients was blended by environmental effects, only genotypic correlation coefficients were partitioned by path analysis to determine the direct and indirect effects of the contributing characters towards seed cotton yield (Table 4). The bold figures on the diagonal represent direct effects and other figures except correlation coefficient with seed cotton yield are indirect effects. The cause and effect of the characters on seed cotton yield with residual effect are displayed in Fig. 1.

The highest positive direct effect (1.982) was exerted by seeds boll\(^{-1}\), followed by single boll weight (1.568) and bolls plant\(^{-1}\), eventually the three characters developed positive and significant correlation with seed cotton yield. [36,51,52,53] reported positive direct effects of bolls plant\(^{-1}\) and single boll weight on seed cotton yield. The highest negative direct effect (-0.872) was paid by unburst bolls plant\(^{-1}\), followed by monopodial branches plant\(^{-1}\) (-0.754). The negative direct effects of the two characters were not counterbalanced and compensated by positive indirect effects remunerated by some seed cotton yield promoting characters, ultimately developed negative and significant correlation with seed cotton yield. The residual effect estimated to 0.0415, suggests 95.85% variation had been encompassed through studying the characters, thereby path analysis was adequate and appropriate.

4. CONCLUSION

Finally, it can be concluded that seed cotton yield is a polygenic complex character, selection of characters without knowing genetic background would not be effective, rather misguides the breeding programs. Twelve characters under study were significantly varied but the mean performance revealed outstanding performance of the genotype, JA-08/D followed by JA-08/C and JA-08/E. Three characters, bolls plant\(^{-1}\), single boll weight and seeds boll\(^{-1}\) exhibited high heritability along with high genetic advance; further these characters were positively and significantly correlated with seed cotton yield. Upon partitioning of genotypic correlation coefficients of eleven characters with seed cotton yield through path analysis, bolls plant\(^{-1}\), single boll weight and seeds boll\(^{-1}\) exerted maximum direct effects to build up positive and significant correlation with seed cotton yield. The negative effects of monopodial branches plant\(^{-1}\) and unburst bolls plant\(^{-1}\) were not balanced by some yield contributing characters. The residual effect was negligible, so maximum portion of variability...
had been exploited in the study. After complete interpretation of results, three genotypes, JA-08/D, JA-08/C and JA-08/E and three characters’ viz., bolls plant-¹, single boll weight and seeds boll-¹ appeared as most outstanding for the improvement of seed cotton yield in upland cotton.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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