Estimation of Heritability, Genes Number and Multivariate Analysis Using Non-segregation and Segregation Generations in Two Cotton Crosses

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This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to evaluate the heritability methods, the genes number equations and comparison between them, as well as multivariate analysis of cotton yield and its components traits in the two crosses G.85 x TNB and G.86 x Suvin. Broad sense heritability (BSH) using Mahmood & Kramer (1951), Burton (1951), Weber & Moorthy (1952), Modified Weber & Moorthy (1952), Briggs & Krowles (1967), Mather & Jinks (1971), Lawrence & Jinks (1973) and Kotecha & Zimmerman (1978) methods, as well as narrow sense heritability using Warner (1952) and Modified Warner (1952) methods were calculated. The methods of BSH and genes number affecting traits were obtained with Chen & Line (1995). The genes number values by equation N3 were much higher than the other equations (close to each other or slightly different) for all studied traits in the two crosses. Based on the ranks method and cluster analysis suggested that there are differences between most the methods of BSH and genes number estimations. The methods of Mather & Jinks

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1. INTRODUCTION

The degree of heritability plays a large role in genetic studies of quantitative traits, including (a) its role in predicting the reliability of the phenotypic value, which is considered evidence of the value of breeding [1], (b) it can be used as an indicator of selection pressure for segregating population [2], (c) it also provides information about the extent to which a private morphogenetic trait can be transmitted to sequential generations, (d) in addition, it helps plant breeders to predict the interaction of the genes in successive generations, (e) so the degree of heritability is necessary for effective breeding programs for all crops [3]. Understanding the differences (variances) and behaviors of the degree of heritability for the yield and yield components is essential for improving such complex/quantitative traits. Heritability degree is defined as “the degree to which a trait is influenced by heredity as compared to the environment” [4].

Two methods of heritability were determined by Lush [5]. One of them is the degree of heritability in the broad sense, which refers to the ratio between the genetic variance of various types (additive, dominance and epistatic variances) and the phenotypic variance. The other method is the degree of heritability in the narrow sense, which is the ratio between the additive genetic variance only and the phenotypic variance. By the degree of heritability in the broad sense, the differences in phenotypes between individuals in a population can be identified through differences in their genotypes. High genetic variation in a crop germplasm pool is a strong indicator of large genetic variability, and the extent or magnitude of this genetic variation within germplasm pool can be determined by estimating heritability in a broad sense among other genetic parameters [6], however, the broad-sense heritability estimate contains some environmental component. Some breeders estimate the environmental component of the total population variance by using non-segregating population since variation in such populations is environmental [3].

There are three main types of heritability estimation in crops i.e. parent-offspring regression, components of variance from ANOVA and approximation of non-heritable variance from genetically uniform populations to estimate total genetic variance [7]. Warner [7] also added that the estimation of non-heritable variance is of special importance in crops like maize where the estimation of heritable variance dependent on the measurement of variance in non-segregating populations as an approximation of non-heritable variance is unsatisfactory. Perhaps due to the large decrease in activity for inbred lines, the total variance in such populations measures the non-heritable variation found only in the low-activity populations, besides it also measures the effects of non-heritable that can be compared to more normal populations. Warner [7] added that it is clear that these weak plants tend to be more susceptible to variations in the environment than the plants with normal activity. On the contrary, using F1 plants to estimate non-heritable variance may provide a value that is too small to
be compared to that in a segregating population if the vigorous genotype tends to be lower, sensitive to environmental fluctuations or too large a value if an environmental variance is associated closely related to the means [7].

Pohlman and Sleper [8] stated that because the number of genes that contribute to variation in a quantitative trait has important implications for development and plant breeding, many plant breeders prefer to estimate the number of genes in a quantitative trait. To estimate the "effective" or "minimum" number to loci segregating, scientists have proposed many statistical methods, including complex and simple methods. The number of gene methods that depend on means and variances between various population groups and their hybrids are considered the simplest and least expensive methods. Within and between population groups, Lande [9] has indicated that estimates of the number of genes that contribute to quantitative trait variances are essential to studying the mechanisms of inheritance and evolution. He also added that the minimum number of genes involved in making a great difference between population groups in a quantitative trait is usually estimated at five or ten genes, with accidental values of up to 20 genes.

Estimates of heritability and the number of genes implicated in expressing quantitative traits are beneficial in the formulation of plant breeding procedures and in assessing the progress of selection. Means and variances of generation of the related population are beneficial in estimating the broad and narrow sense heritability as well as the minimum number of genes affecting the quantitative trait in a hybrid. Due to the difference in the calculation of environmental variance, the various methods for estimating the heritability and the number of genes for the quantitative traits in crops have differed [3]. The objectives of this study were 1) to study the relationships, similarities and dissimilarities among the P1, P2, F1, F2, BC1 and BC2 generations 2) to compare various methods of estimates heritability and 3) to determine genes number affecting yield and yield components traits in two cotton crosses.

2. MATERIALS AND METHODS

2.1 Genetic Material and Field Procedure

The experiments were carried out during the three successive seasons from 2018 to 2020 at Sakha Agricultural Research Station at Kafr El-Shiekh Governorate. The genetic materials used in this study were the two Egyptian varieties Giza 85 (G. 85) and Giza 86 (G. 86) as well as TNB (Australian) and Suvin (Indian) genotypes. All these genotypes belong to Gossypium barbadense, L. In 2018 season, the four parental varieties were crossed to produce F1 hybrid seeds for the two crosses G. 85 x TNB and G.86 x Suvin. At 2019 season, each F1 was backcrossed to both parents to obtain BC1 and BC2, the parents were also crossed for more hybrid seeds and the F1 plants was selfed to obtain F2 seeds. The six populations i.e. P1, P2, F1, F2, BC1 and BC2 for the two crosses were evaluated separately in a randomized complete blocks design (RCBD) with three replications during 2020 season. Each replicate consisted of 10 rows for F2, 5 rows for BC1 and BC2 crosses (segregating generations), and 3 rows for each non-segregating generations P1, P2 and F1. Each row 4 meters in length and 0.60 m in width as well as comprised 10 hills. Hills were spaced at 40 cm apart and thinned to one plant per hill. All recommended agronomic practices of cotton were applied from sowing to harvesting to get a good and healthy plant population as usual.

2.2 Traits Measurement

The studied traits on an individual cotton plant basis were taken of the six non- segregation and segregation populations in the two crosses studied. The data were recorded for boll weight in grams (B.W, g), seed cotton yield/plant in grams (S.C.Y./P, g), lint cotton yield/plant in grams (L.C.Y./P, g), lint percentage (L%), number of bolls/plant (No. of B./P) and seed index (S.I, g) traits. All guarded plants were harvested to measure of these traits.

2.3 Statistical and Genetic Procedures

The analysis of variance (ANOVA) was performed for studied traits in six populations, and computed according to the method of Gomez and Gomez [10]. Heritability can be estimated in several ways from data in a six parameters. Heritability estimates in broad sense were calculated using the methods proposed by Mahmud & Kramer [11], Burton [12], Weber & Moorthy [13], Modified Weber & Moorthy [13], Briggs & Krowles [14], Mather & Jinks [15], Lawrence & Jinks [16] and Kotecha & Zimmerman [17] (Table 1). The narrow sense heritability was calculated according to methods of Warner [7] and Modified Warner [7]. The heritability estimates were categorized as
3.1 Analysis of Variance

Traits among non-heritability and number of genes estimates were calculated according to Pesek and Baker [20] and Lande [9] method, respectively. Principal component and cluster analysis were done using a computer software program PAST version 2.17c.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Analysis of variance (ANOVA) for six studied traits among non-segregation (P1, P2 and F1) and segregation (F2, BC1 and BC2) generations are offered in Table 3. The mean squares due to segregation (P1, P2 and F1) and generations variances, respectively.

Table 1. The equations used for the estimation of heritability in this study

| Methods                          | Numerator                          | Denominator                          | Environmental Variance (V_e) |
|----------------------------------|------------------------------------|--------------------------------------|------------------------------|
| Mahmud & Kramer                  | V_{F_2} - V_e                      | V_{F_2}                              | V_{P_1}xV_{F_2}              |
| Burton                           | V_{F_2} - V_e                      | V_{F_2}                              | V_{F_1}                       |
| Weber & Moorthy                  | V_{F_2} - V_e                      | V_{F_2}                              | V_{P_1}xV_{P_2}xV_{F_1}      |
| Modified Weber & Moorthy         | V_{F_2} - V_e                      | V_{F_2}                              | V_{P_1}xV_{P_2}xV_{F_1}      |
| Briggs & Knowles                 | V_{F_2} - V_e                      | V_{F_2}                              | V_{F_1}                       |
| Mather & Jinks                   | V_{F_2} - V_e                      | V_{F_2}                              | (\sigma^2 P_2 + \sigma^2 P_2 + 2\sigma^2 F_1)/4 |
| Lawrence & Jinks                 | V_{F_2} - V_e                      | V_{F_2}                              | 0.25(\sigma^2 P_1 + \sigma^2 P_2 + 2\sigma^2 F_1) |
| Kotecha & Zimmerman             | V_{F_2} - V_e                      | V_{F_2}                              | (V_{P_1} + V_{P_2} + V_{F_1})/3 |

\[ V_{P_1} = \text{First parent variance}, V_{P_2} = \text{Second parent variance}, V_{F_1} = \text{First generation variance}, V_{F_2} = \text{Second generation variance}, V_{BC_1} = \text{First back cross variance}, V_{BC_2} = \text{Second back cross variance}, V_e = \text{Environmental variance.} \]

Table 2. The equations used for the estimation of the number of genes in this study

| Methods  | Numerator                          | Denominator                          |
|----------|------------------------------------|--------------------------------------|
| N1       | \((P_2 - \bar{P}_1)^2\)            | 8(V_{F_2} - V_{F_1})                 |
| N2       | \((P_2 - \bar{P}_1)^2\)            | 8([V_{F_2} - 0.25V_{P_1} + 0.25V_{P_2} + 0.5V_{F_1}]\] |
| N3       | \((P_2 - \bar{P}_1)^2\)            | 8([V_{F_2} - (V_{BC_1} + V_{BC_2})]\] |
| N4       | \((P_2 - \bar{P}_1)^2\)            | 8([V_{BC_1} + V_{BC_2}] - 0.5V_{P_1} + V_{P_2} + V_{F_1}]\] |
| N5       | \((\bar{P}_1 - \bar{P}_1)^2\)     | 4(V_{BC_1} - 0.5(V_{F_1} + V_{P_1})\] |
| N6       | \((P_2 - \bar{F}_1)^2\)            | 4(V_{BC_2} - 0.5(V_{F_1} + V_{P_2})\] |

\[ P_1, P_2 \text{ and } \bar{F}_1: \text{First parent, Second parent and First generation means, respectively.} \]

Suggested by Robinson et al. [18] (0-30% = low; 31-60% = moderate; above 60% = high). The number of genes controlling the trait was estimated using the six equations suggested by Chen and Line [19] (Table 2). Standard errors for heritability and number of genes estimates were calculated according to Pesek and Baker [20] and Lande [9] method, respectively. Principal component and cluster analysis were done using a computer software program PAST version 2.17c.
significant differences between genotypes for yield and its components traits in cotton. The CVs values displayed that the environmental influence was large for boll weight and seed index traits and was lower for other studied traits. The magnitude of CV% indicated that the generations had exploitable genetic variability during selection of traits examined in cotton. The CV% values were high for yield and its components traits in cotton with similar results reported by Raza et al. [24] and Li et al. [25].

3.2 Mean Performances

Significant differences among six generations were found for all studied traits in the two crosses, indicating the presence of genetic variability for these traits in the study materials (Table 4). The performance of G. 85 and G. 86 varieties were higher than TNB and Suvin genotypes for all studied traits, except for boll weight (TNB) and seed index (Suvin) traits. The F1 performance was higher than the respective parents, F2, BC1 and BC2 generations for all the studied traits in the two crosses. The relation between non-segregation and segregation generations revealed that there was different behavior for studied traits in the study materials. Thus, it is possible to benefit from the selection in the segregation generations in future breeding programs of improving these traits in Egyptian cotton.

3.3 Variance Components and Heritability

Environmental variance \( V_E \), genetic variance \( V_G \) and heritability were calculated using different methods based on six population's variances for the studied traits at the two crosses and given in Tables 5 and 6. Estimates of various studied methods of BSH for all studied traits exhibited highly significant difference \( (P < 0.01) \) in the two crosses. This conformed to the earlier findings of El-Hashash [3] in cotton. The results of the studied methods showed that the lowest value of \( V_E \) corresponded to the largest value of \( V_G \) and BSH, and vice versa. The highest values of the \( V_E \) and BSH as well as the lowest values of \( V_G \) were registered by Mahmood & Kramer methods for boll weight in the two crosses, for lint percentage in the cross G.85 x TNB as well as for number of bolls/plant and seed index traits in the cross G.86 x Suvin; by Burton method for seed cotton yield/plant and lint percentage traits in the cross G.86 x Suvin, for lint cotton yield/plant in the two crosses as well as for number of bolls/plant and seed index traits in the cross G.85 x TNB; and by Modified Weber & Moorthy method for seed cotton yield/plant in the cross G.85 x TNB. The Modified Weber & Moorthy method for boll weight, number of bolls/plant and seed index traits in the two crosses; the Briggs & Knowles method for seed cotton yield/plant and lint percentage traits in the cross G.86 x Suvin as well as lint cotton yield/plant in the two crosses; and the Burton method for seed cotton yield/plant and lint percentage traits in the cross G.85 x TNB were recorded the minimum values of \( V_E \) and BSH as well as the maximum values of \( V_G \). The values of BSH by all studied methods in the cross G.85 x TNB were higher than in the cross G.86 x Suvin for boll weight, seed and lint cotton yields/plant, lint percentage and seed index traits, except seed cotton yield/plant and lint percentage with Burton, Mather & Jinks and Lawrence & Jinks methods; for lint percentage with Modified Weber & Moorthy method; as well as for seed index with Mahmood & Kramer and Briggs & Knowles methods. While, the cross G.86 x Suvin was

| Table 3. The mean squares for studied traits among non-segregation and segregation generations in two crosses of cotton |
| --- |
| **Crosses** | **S.O.V** | **df** | **Mean Squares of Traits** |
| | | | **B.W.** | **S.C.Y./P** | **L.C.Y./P** | **L.%** | **No.B./P** | **S.I.** |
| G. 85 x TNB | Generations | 5 | 0.17 | 607.97** | 122.19** | 2.66 | 39.06** | 2.94 |
| G.86 x Suvin | Generations | 5 | 0.36 | 1946.20** | 302.77** | 3.31 | 93.08** | 1.59 |

B.W.: Boll weight; S.C.Y./P: Seed cotton yield/plant; L.C.Y./P: Lint cotton yield/plant; L.%: Lint percentage; No.B./P: Number of bolls/plant; S.I.: Seed index; *and**: Significant at \( P = 0.05 \) and 0.01, respectively.
### Table 4. Means (\(\bar{x}\)) and standard errors (SE) for studied traits in four cotton genotypes and their F1, F2, BC1 and BC2

| Populations | Traits | B.W. | S.C.Y./P | L.C.Y./P | L.% | No. B./P | S.I. |
|-------------|--------|------|---------|----------|-----|---------|------|
|             |        | I    | II      | I        | II  | I       | II   |
| P1          | \(\bar{x}\) | 3.271| 3.741   | 78.474   | 90.459| 35.307  | 37.702| 37.832| 37.728| 24.480| 28.575| 9.445| 9.567|
|             | SE     | 0.437| 0.394   | 1.838    | 1.247| 0.964   | 1.028| 0.278 | 0.663 | 0.563 | 0.173 | 0.467 | 0.346|
| P2          | \(\bar{x}\) | 3.609| 3.144   | 76.279   | 88.376| 32.523  | 36.184| 36.897| 36.865| 23.554| 27.881| 9.902| 8.524|
|             | SE     | 0.239| 0.301   | 1.341    | 1.194| 0.838   | 0.867| 0.219 | 0.804 | 0.315 | 0.149 | 0.493 | 0.289|
| F1          | \(\bar{x}\) | 3.705| 3.908   | 111.725  | 147.272| 42.467  | 57.554| 38.673| 39.880| 30.169| 37.351| 10.446| 9.948|
|             | SE     | 0.392| 0.372   | 2.034    | 0.736| 0.708   | 0.692| 0.461 | 0.505 | 0.353 | 0.161 | 0.412 | 0.391|
| F2          | \(\bar{x}\) | 3.313| 3.034   | 77.429   | 83.328| 32.717  | 30.758| 36.067| 38.638| 24.282| 25.906| 7.729 | 8.389|
|             | SE     | 0.723| 0.675   | 6.229    | 3.663| 2.912   | 2.782| 1.076 | 2.062 | 0.881 | 0.438 | 1.255 | 0.845|
| BC1         | \(\bar{x}\) | 3.328| 3.230   | 96.985   | 86.004| 36.278  | 33.609| 37.099| 38.393| 25.584| 24.015| 8.058 | 8.065|
|             | SE     | 0.574| 0.476   | 4.159    | 2.581| 1.925   | 1.937| 0.736 | 1.333 | 0.646 | 0.379 | 0.832 | 0.585|
| BC2         | \(\bar{x}\) | 3.066| 3.324   | 82.182   | 79.700| 22.966  | 31.119| 36.510| 37.523| 18.945| 21.010| 8.733 | 8.967|
|             | SE     | 0.439| 0.476   | 4.634    | 2.597| 2.175   | 1.995| 0.784 | 1.572 | 0.596 | 0.215 | 0.939 | 0.606|

*B.W.: Boll weight; S.C.Y./P: Seed cotton yield/plant; L.C.Y./P: Lint cotton yied/plant; L.%: Lint percentage; No. B./P: Number of bolls/plant; S.I.: Seed index; I: G 85 x TNB; II: G 86 x Suvin.

### Table 5. Estimates of environmental (\(V_E\)) and genetic (\(V_G\)) variances using various methods for studied traits in the two crosses

| Methods            | Traits | B.W. | S.C.Y./P | L.C.Y./P | L.% | No. B./P | S.I. |
|--------------------|--------|------|---------|----------|-----|---------|------|
|                    |        | \(V_E\) | \(V_G\) | \(V_E\) | \(V_G\) | \(V_E\) | \(V_G\) | \(V_E\) | \(V_G\) | \(V_E\) | \(V_G\) | \(V_E\) | \(V_G\) |
| Mahmud & Kramer     | I      | 0.313 | 1.256   | 7.394    | 108.990| 2.424   | 23.014| 0.183 | 3.293 | 0.532 | 1.795 | 0.691 | 4.037|
|                    | II     | 0.357 | 1.101   | 4.469    | 35.786| 2.672   | 20.548| 1.599 | 11.160| 0.077 | 0.499 | 0.299 | 1.845|
| Burton             | I      | 0.460 | 1.109   | 12.406   | 103.979| 1.504   | 23.934| 0.637 | 2.839 | 0.373 | 1.954 | 0.509 | 4.129|
|                    | II     | 0.416 | 0.952   | 4.159    | 38.361| 1.437   | 21.783| 0.766 | 11.993| 0.078 | 0.499 | 0.459 | 1.685|
| Weber & Moorthy    | I      | 0.356 | 1.213   | 8.786    | 107.598| 2.067   | 23.371| 0.277 | 3.198 | 0.473 | 1.855 | 0.624 | 4.104|
|                    | II     | 0.375 | 0.993   | 3.190    | 37.066| 2.123   | 21.047| 1.251 | 11.508| 0.077 | 0.499 | 0.345 | 1.799|
| Mod.Weber & Moorthy| I      | 0.548 | 1.021   | 6.069    | 110.316| 2.050   | 23.388| 0.454 | 3.021| 0.678 | 1.649 | 0.835 | 3.893|
|                    | II     | 0.570 | 0.798   | 2.838    | 37.417| 2.129   | 21.092| 1.407 | 11.352| 0.174 | 0.402 | 0.536 | 1.609|
| Briggs & Knowles   | I      | 0.372 | 1.197   | 7.764    | 108.621| 2.448   | 22.990| 0.188 | 3.288 | 0.624 | 1.703 | 0.692 | 4.036|
|                    | II     | 0.370 | 0.998   | 4.474    | 35.782| 2.711   | 20.509| 1.629 | 11.130| 0.078 | 0.499 | 0.304 | 1.840|
| Mather & Jinks     | I      | 0.416 | 1.153   | 10.085   | 106.300| 1.976   | 23.462| 0.412 | 3.063 | 0.499 | 1.829 | 0.600 | 4.127|
|                    | II     | 0.393 | 0.975   | 3.049    | 37.206| 2.074   | 21.146| 1.197 | 11.562| 0.078 | 0.499 | 0.382 | 1.763|
| Methods               | Traits       | B.W. | S.C.Y./P | L.C.Y./P | L.% | No. B/P | S.I. |
|-----------------------|--------------|------|----------|----------|-----|---------|------|
|                       | Crosses      | V_E  | V_G      | V_E      | V_G | V_E      | V_G  |
| Lawrence & Jinks      | I            | 0.416| 1.153    | 10.085   | 106.300| 1.976    | 23.462| 0.412    | 3.063| 0.499    | 1.829| 0.600    | 4.127|
|                       | II           | 0.393| 0.975    | 3.049    | 37.206 | 2.074    | 21.146| 1.197    | 11.562| 0.078    | 0.499| 0.38     | 1.763|
| Kotecha & Zimmerman   | I            | 0.402| 1.168    | 9.311    | 107.074| 2.133    | 23.305| 0.337    | 3.138| 0.541    | 1.787| 0.631    | 4.097|
|                       | II           | 0.385| 0.983    | 3.524    | 36.732 | 2.287    | 20.934| 1.341    | 11.418| 0.078    | 0.499| 0.356    | 1.789|
| Warner                | I            | 1.570| 114.444  | 25.567   | 0.384  | 2.339    | 4.732 |
|                       | II           | 1.376| 40.291   | 23.243   | 12.771 | 0.583    | 2.159 |
| Mod. Warner           | I            | 1.270| 115.699  | 25.292   | 3.262  | 1.912    | 4.248 |
|                       | II           | 1.013| 40.005   | 22.920   | 12.181 | 0.390    | 1.832 |

B.W.: Boll weight; S.C.Y./P: Seed cotton yield/plant; L.C.Y./P: Lint cotton yield/plant; L.%: Lint percentage; No.B./P: Number of bolls/plant; S.I.: Seed index; I: G.85 x TNB; II: G.86 x Suvin.

Table 6. Estimates of Broad (h²_b) and narrow (h²_n) sense heritability as well as standard errors (SE) using various methods for studied traits in the two crosses.
## Methods

| Traits | B.W. | S.C.Y./P | L.C.Y./P | L.% | No. B./P | S.I. |
|--------|------|----------|----------|-----|----------|------|
| Crosses | I | II | I | II | I | II | I | II | I | II | I | II | I | II | I | II |
| SE | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| $h_r^2$ | 0.808** | 0.740** | 0.819** | 0.994** | 0.987** | 0.939** | 0.955** | 0.821** | 0.676** | 0.899** | 0.854** |
| SE | 0.012 | 0.016 | 0.005 | 0.000 | 0.001 | 0.004 | 0.003 | 0.012 | 0.019 | 0.007 | 0.010 |

**B.W.: Boll weight; S.C.Y./P: Seed cotton yield/plant; L.C.Y./P: Lint cotton yield/plant; L.%: Lint percentage; No.B./P: Number of bolls/plant; S.I.: Seed index; I: G.85 x TNB; II: G.86 x Suvin; *and**: Significant at P = 0.05 and 0.01, respectively**

### Table 7. Ranks of broad sense heritability methods using all studied traits in the two crosses

| BSH Methods | B.W. | S.C.Y./P | L.C.Y./P | L.% | No. B./P | S.I. | R | $\sigma^2$ | SDR | RS |
|--------------|------|----------|----------|-----|----------|------|---|---------|------|---|
| Mahmud & Kramer | 1 | 1 | 2 | 7 | 7 | 1 | 7 | 5 | 1 | 6 | 1 | 3.83 | 7.47 | 2.73 | 6.57 |
| Burton | 7 | 7 | 8 | 1 | 1 | 1 | 8 | 1 | 1 | 3 | 1 | 7 | 3.83 | 9.47 | 3.08 | 6.91 |
| Weber & Moorthy | 2 | 3 | 4 | 5 | 4 | 5 | 3 | 4 | 2 | 1 | 4 | 3 | 3.33 | 1.39 | 1.18 | 4.51 |
| Mod.Weber & Moorthy | 8 | 8 | 1 | 2 | 4 | 7 | 6 | 8 | 8 | 8 | 8 | 6.00 | 6.17 | 2.48 | 8.48 |
| Briggs & Knowles | 3 | 2 | 3 | 7 | 8 | 8 | 2 | 8 | 7 | 3 | 6 | 2 | 4.92 | 6.24 | 2.50 | 7.42 |
| Mather & Jinks | 5 | 5 | 6 | 3 | 2 | 2 | 5 | 2 | 3 | 3 | 2 | 5 | 3.58 | 2.08 | 1.44 | 5.02 |
| Lawrence & Jinks | 5 | 5 | 6 | 3 | 2 | 2 | 5 | 2 | 3 | 3 | 2 | 5 | 3.58 | 2.08 | 1.44 | 5.02 |
| Kotecha & Zimmerman | 4 | 4 | 5 | 6 | 6 | 6 | 4 | 5 | 6 | 3 | 5 | 4 | 4.83 | 0.97 | 0.99 | 5.82 |

**B.W.: Boll weight; S.C.Y./P: Seed cotton yield/plant; L.C.Y./P: Lint cotton yield/plant; L.%: Lint percentage; No.B./P: Number of bolls/plant; S.I.: Seed index; I: G.85 x TNB; II: G.86 x Suvin; R: Rank mean; $\sigma^2$: variance; SDR: standard deviation of ranks; RS: rank sum**
The numerator and denominator in broad sense heritability (BSH) formula are an estimate of the total genetic \( V_G = V_e - V_E \) and phenotypic \( V_P \) variances, respectively (Table 1). The estimations methods of BSH by Mahmud & Kramer and Briggs & Krowles were calculated using two parents and F2 generation variances. While, the methods of estimations according to Lawrence & Jinks, Weber & Moorthy, Modified Weber & Moorthy and Kotecha & Zimmerman using the two parents, F1 and F2 generations variances. The BSH had measured using the variances of F1 and F2 generations according to Burton method. The estimating BSH based on the estimation of genetic variances components as outlined by Mather & Jinks method [3]. The methods of Mather & Jinks and Lawrence & Jinks gave the same results of the \( V_E \) and BSH for all studied traits in the two crosses, because calculated \( V_E \) by these two methods were equal. In addition, the two methods of Mahmud & Kramer and Briggs & Krowles as well as the two methods Weber & Moorthy and Kotecha & Zimmerman were showed equal or close values for the studied traits in the two crosses, because the \( V_E \) by Mahmud & Kramer and Briggs & Krowles methods as well as Weber & Moorthy and Kotecha & Zimmerman methods were calculated using the two parent’s variances as well as the two parent’s and F1 variances, respectively. On the other side, the other studied methods were differed for all studied traits in the two crosses. These results agreed with the view by El-Hashash [3] in cotton, which the BSH estimates using these methods were different or fairly consistent or equal for each studied trait in the two studied crosses. While, the Mahmud & Kramer, Briggs & Krowles and Lawrence & Jinks methods of BSH estimates were fairly consistent for each trait in summer rape [27].

El-Hashash [3] mentioned that, these BSH methods include to the same denominator \( (V_F) \) but the numerator \( (V_G = V_F - V_E) \) is different, thus BSH values were different because these methods were different in calculated the \( V_E \). The \( V_E \) had estimated by \( \sqrt{\frac{V_P}{V_G}} \) (Mahmud & Kramer), by \( V_F \) (Burton), by \( \frac{1}{\sqrt{V_P \times V_G}} \) (Weber & Moorthy), by \( \frac{1}{\sqrt{V_{F1} \times V_{F2} \times V_{F1}}} \) (Modified Weber & Moorthy), by \( \frac{1}{\sqrt{V_{F1} \times V_{F2}}} \) (Briggs & Krowles), by \( \frac{1}{\sqrt{V_{F1} + V_{F2}}} \) (Briggs & Knowles), by \( \frac{(\sigma^2 \times V_{F1} + \sigma^2 \times V_{F2} + 2 \sigma^2 \times V_{F1})}{4} \) (Mather & Jinks and Lawrence & Jinks) and by \( \frac{(V_{F1} + V_{F2} + V_{F2})}{3} \) (Kotecha & Zimmerman). The BSH numerator can be influenced by the \( V_E \) (environment), because the \( V_E \) expression can be influenced with the environment [3,29], this is called genotype by environment interaction, there is not one degree of heritability for a given trait in a given species, because heritability can and often does differ among populations and among environments [29]. El-Hashash [3] stated that, the differences between the methods of BSH estimations due to (1) type and number of generations variances used for calculation of the \( V_E \) and (2) equation of \( V_E \), therefore, the estimated values of BSH varied according the calculation of various methods.

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greater than the cross G.85 x TNB for number of bolls/plant by all BSH methods, except Modified Weber & Moorthy method. The highest values by Burton method [26] and by Mahmud & Kramer and Burton methods and the lowest values by Modified Weber & Moorthy methods [3] of estimating BSH were recorded for the for most traits in cotton. According to Robinson et al. [18], the results of all BSH methods exhibited the highest values for all traits (BSH > 0.60) in the two crosses, except Modified Weber & Moorthy method for seed index boll weight in the cross G.86 x Suvin (BSH < 0.60). High BSH values are due to high \( V_G \) and low \( V_E \) values, thus, reducing the \( V_E \) generally increases the BSH [3].

El-Hashash [3] mentioned that, these BSH values by Warner method were higher than by Modified Warner method for all studied traits in the two crosses (Tables 5 and 6). The values of BSH by Warner and Modified Warner methods were highly significant and recorded the highest values (BSH > 0.60) for all studied traits in the two crosses. These results are in agreement with El-Hashash [3]. The difference between these methods due to the method of calculating \( V_G \) (numerator), where an increase in \( V_G \) leads to an increase in BSH, and vice versa [3]. The BSH is a reflection of the amount of additive, fixable, heritable variation. Warner’s method requires the additional assumption that environmental component of variation of the F2 and backcross generations are also of comparable magnitude [27]. Hussain et al. [21] mentioned that NSH calculated by Warner method for seed cotton yield (0.71) was high, suggesting the chances for improving these character through selection from the segregating populations. In most cases, the NSH values decreased, due to the opposite trends of dominance and dominance x dominance effects resulting in reduced overall dominance variance [28].
For comparison and determination of the similarities and differences between BSH methods on the basis of based on six studied traits in the two crosses, the ranks method and the cluster analysis with Ward method were used. Ranking method exhibited the estimates of BSH based on a single method were contradictory for all studied traits, except Mather & Jinks and Lawrence & Jinks methods (Table 7). The estimates of HBS based on a single method were contradictory for all studied traits, except Mather & Jinks and Lawrence & Jinks methods. According to ranks of BSH methods using all studied traits in the two crosses, the ranks of Mather & Jinks and Lawrence & Jinks methods were identical for all studied traits in the two crosses. On the other hand, the other methods differed in estimating of BSH for all the studied traits. Based on the rank mean, standard deviation and rank sum of rank, the HBS values were identical by Mather & Jinks and Lawrence & Jinks methods, almost similar or close by Mahmud & Kramer and Briggs & Knowles as well as close by Weber & Moorthy and Kotecha & Zimmerman methods. While, Burton and Mod.Weber & Moorthy methods were showed different in estimates of HBS for studied traits. According to the cluster analysis (multivariate statistical technique), the estimations of BSH methods were divided into two groups (Fig. 1). The first group (A) consisted of Modified Weber & Moorthy method, while, the second group (B) comprised of the other methods. The second group contained four clusters. The first, second, third and fourth clusters consists of Burton method, Mahmud & Kramer and Briggs & Krowles methods, Mather & Jinks and Lawrence & Jinks methods as well as Kotecha & Zimmerman and Weber & Moorthy methods, respectively. The tree diagram had exhibited the

![Fig. 1. Tree diagram for eight methods of estimates BSH using ward's method](image)
minimum distance or dissimilarity between the BSH methods inside each cluster. While, the highest distance of BSH methods were found among the Modified Weber & Moorthy method and the others methods as well as among the four clusters. The estimates of BSH methods were showed differences for various traits in cotton by El-Hashash [3], in summer rape by Gram et al. [27], in wheat by Heydari et al. [30] as well as in corn by Schmidt [31] and Dorri et al. [32]. Gram et al. [27] stated that Mahmud & Kramer, Briggs & Krowles and Lawrence & Jinks methods of BSH estimates were fairly consistent for each trait in summer rape. Schmidt [31] reported that, the methods of estimating BSH in corn were not significantly different from each other in most comparisons; when significant differences did occur, however, they were most often between comparisons of Burton's method with Mahmud & Kramer, Weber & Moorthy and modified Weber & Moorthy methods.

The high values of BSH indicate the preponderance of dominance variance in governing the studied traits. Also, the $V_g$ more than the $V_e$ and plays a major part in total variance and will make the selection process more effective for genetic improvement of these traits. While, low values of BSH indicate that $V_e$ is of importance or environmental factors strongly influence trait and breeding for improving such trait is difficult. When BSH values are high, the genetic gain of the cotton yield can be achieved through the practice of individual plant selection in the early generations, while when BSH values are low the selection of the higher yield in cotton can be based on replicated plot experiments at more than one site in the more advanced generations [3]. El-Hashash [33] reported that, the values of BSH were high for yield and yields components traits in single and double-cross hybrids. High NSH estimates by Warner's method were obtained for number of bolls/plant, seed cotton yield/plant, lint percentage and plant height characters [34]. Khan et al. [35] stated that lower values of the estimates of BSH and NSH indicate that the effects of $V_e$ error were greater than the effects of the genetic components. In Upland cotton, BSH values ranged from moderate to high in quantity for yield and their components traits among F4 populations [24].

3.4 The Minimum Number of Genes

The values of number of effective factors controlling quantitative studied traits (Mendelian genes or quantitative trait loci) were estimated using six equations by Chen and Line [19] and are shown in Table 8. The six equations to the estimates of genes number were showed significant or highly significant for lint cotton yield/plant and lint percentage traits in the cross G.85 x TNB, for seed index trait in the cross G.86 x Suvin and for number of bolls/plant trait in the two crosses. These results are in good agreement with those reported by El-Hashash [3]. The values of the genes number controlling all the traits in the two crosses under study by equation N3 ($N>2$) were much higher than the other equations ($N<1$). The number of genes estimated by other equations was close to each other or slightly different. But, these equations were far apart or completely different from the N3 equation, which were differed in estimating of genes number controlling in these traits. The number of genes by the N3 equation in the cross G.85 x TNB was higher than in the cross G.86 x Suvin for boll weight, lint percentage and number of bolls/plant traits, while, the cross G.86 x Suvin had greater than the cross G.85 x TNB for other studied traits. These results corroborates the results obtained by Sofalian et al. [36] who mentioned that the equation N3 provided the maximum estimates of gene number in wheat. El-Hashash [3] stated that the methods values of genes number were differed for all studied traits, also he added, multiple genetic factors were involved in controlling for most traits in cotton. Using N2, N3 and N4 equations as well as other methods, the number of effective factors had larger or less than one for traits in watermelon [37].

All of the equations agree that the numerator is the deviation square between the two non-segregating populations (P1, P2 and F1). It is clear from Table 2 that equations N1, N2, N3 and N4 have the same numerator, but the numerators are different in both equations N5 and N6. While, the denominator differed in the six equations, which depend on the variances of the segregating and non-segregating populations, according to each equation. Since difference between the parents is a common field in the equations, the validity of the estimates is specific to each given experiment [27]. Methods for estimating the number of genes depend on the variability and the means, where the highest values of genes number may depend on the higher variability and lower means. These estimates should be considered only indicative [37].
| Equations | Traits | Crosses | N     | SE    | N     | SE    | N     | SE    | N     | SE    | N     | SE    | N     | SE    | N     | SE    |
|-----------|--------|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| N₁        | I      | B.W.    | 0.013 | 0.013 | 0.006 | 0.004 | 0.040**| 0.013 | 0.038**| 0.011 | 0.055*| 0.027 | 0.006 | 0.006 |       |
|           | II     | S.C.Y./P| 0.047 | 0.030 | 0.014 | 0.008 | 0.013 | 0.008 | 0.008 | 0.006 | 0.120**| 0.034 | 0.081**| 0.028 |       |
| N₂        | I      | L.C.Y./P| 0.012 | 0.012 | 0.006 | 0.004 | 0.041**| 0.013 | 0.036**| 0.010 | 0.059*| 0.029 | 0.006 | 0.006 |       |
|           | II     | L. %    | 0.046 | 0.029 | 0.015 | 0.008 | 0.014 | 0.008 | 0.008 | 0.006 | 0.120**| 0.034 | 0.077**| 0.026 |       |
| N₃        | I      | No.B./P | 9.546 | 9.394 | 10.211| 6.929 | 7.657**| 2.469 | 13.396**| 3.891 | 9.581*| 4.792 | 6.301 | 6.016 |       |
|           | II     | S.I.    | 5.501 | 3.482 | 15.289| 8.293 | 12.551| 7.262 | 7.615 | 5.920 | 8.886**| 2.494 | 9.613**| 3.276 |       |
| N₄        | I      | B.W.    | 0.022 | 0.022 | 0.006 | 0.004 | 0.048**| 0.015 | 0.043**| 0.012 | 0.092*| 0.046 | 0.008 | 0.008 |       |
|           | II     | S.C.Y./P| 0.102 | 0.064 | 0.017 | 0.009 | 0.016 | 0.009 | 0.010 | 0.008 | 0.158**| 0.044 | 0.110**| 0.037 |       |
| N₅        | I      | L.C.Y./P| 0.007 | 0.007 | 0.032 | 0.022 | 0.046**| 0.015 | 0.035**| 0.010 | 0.142*| 0.071 | 0.003 | 0.003 |       |
|           | II     | L. %    | 0.003 | 0.002 | 0.137 | 0.075 | 0.084 | 0.048 | 0.018 | 0.014 | 0.000**| 0.000 | 0.004**| 0.001 |       |
| N₆        | I      | No.B./P | 0.012 | 0.012 | 0.023 | 0.016 | 0.033**| 0.011 | 0.028**| 0.008 | 0.114*| 0.057 | 0.003 | 0.002 |       |
|           | II     | S.I.    | 0.002 | 0.001 | 0.134 | 0.073 | 0.074 | 0.043 | 0.013 | 0.010 | 0.001**| 0.000 | 0.003**| 0.001 |       |

B.W.: Boll weight; S.C.Y./P: Seed cotton yield/plant; L.C.Y./P: Lint cotton yield/plant; L. %: Lint percentage; No.B./P: Number of bolls/plant; S.I.: Seed index; I: G.85 x TNB; II: G.86 x Suvin; *and**: Significant at P = 0.05 and 0.01, respectively.
Table 9. Ranks of genes number equations using all studied traits in the two crosses

| Equations | B.W. | S.C.Y./P | L.C.Y./P | L.% | No. B/P | S.I. | $\bar{R}$ | $\sigma^2$ | SDR | RS |
|-----------|------|----------|----------|-----|---------|------|---------|---------|------|-----|
| N1        | 3    | 3        | 4        | 6   | 5       | 6    | 3       | 5       | 6    | 3   |
| N2        | 4    | 4        | 4        | 5   | 4       | 5    | 3       | 4       | 1.17 | 0.47|
| N3        | 1    | 1        | 1        | 1   | 1       | 1    | 1       | 1       | 1.00 | 0.00|
| N4        | 2    | 2        | 4        | 2   | 4       | 4    | 2       | 2       | 2.83 | 0.97|
| N5        | 6    | 5        | 2        | 2   | 3       | 2    | 5       | 2       | 6    | 5   |
| N6        | 4    | 6        | 3        | 3   | 6       | 3    | 6       | 3       | 5    | 6   |

B.W.: Boll weight; S.C.Y./P: Seed cotton yield/plant; L.C.Y./P: Lint cotton yield/plant; L.%: Lint percentage; No.B./P: Number of bolls/plant; S.I.: Seed index; I: G.85 x TNB; II: G.86 x Suvin; $\bar{R}$: Rank mean; $\sigma^2$: variance; SDR: standard deviation of ranks; RS: rank sum.

Fig. 2. Dendrogram for six equations of estimates genes number using ward’s method.

The ranks method and cluster analysis were used for comparing among the equations of the genes number studied on the basis of studied traits in the two crosses. According to ranks method, the ranks of third equation of the genes number for all studied traits in the two crosses were identical (Table 9). While, ranks of the other equations were exhibited different in estimating the genes number for all studied traits in the two crosses. According to the rank mean, standard deviation and rank sum of rank, the N3 equation had the highest values of genes number, followed by N4, N1, N2, N5 and N6 equations. The six equations were classified into three clusters by separate cluster analysis with Ward method (Fig. 2). The first cluster consists of N3 equation, which had given the highest values of genes number. The equations N5 and N6 were classified as the second cluster. The third cluster includes the N4, N1 and N2 equations. The first and second groups showed the lowest distance or difference among the equations within them.
thus these equations were highly similar of estimating gene number for all studied traits in the two crosses. While, high distances were found between the N3 equation and other equations in the second and third clusters, consequently there were differences in estimating the genes number by the N3 equation and the other equations. Although the estimates of the different formulas may be subject to their inherent assumptions, it is observed that the estimated numbers of all equations were close together, with the exception of equation N3 [36]. For many geneticists, inherited traits quantitatively refer to a large number of genes each with small effects, thus, methods for accurately estimating the number of genes is generally difficult and not pursued [3].

3.5 Principal Component Analysis

Principal component analysis (PCA) is a multivariate statistical technique. The PCA simplifies the complex data by transforming the number of correlated variables into a smaller number of variables called principal components. Hence, it has been used to understand the relationship between the six generations and the studied traits. The first main PC1 extracted had eigenvalues larger than one (Eigen value >1) with values 4.24 and 4.78 in the two crosses G.85 x TNB and G.86 x Suvin, respectively (Table 10). While, the other PCs extracted had eigenvalues less than one (Eigen value < 1). The first two PCs explained 86.29% and 92.36% of the total variation in the original variables in the two crosses G.85 x TNB and G.86 x Suvin, respectively. Based on all studied traits, the PC1 and PC2 had mainly distinguished the generations in different groups. Thus, the PC1 and PC2 were employed to draw a biplot (Fig. 3). According to Isong et al. [38], Rathinavel [39], Akter et al. [40] and Vinodhana and Gunasekaran [41] the eigen values had higher than one for the first six, eight, seven and five, PCAs, and which contributed for 70.39%, 83.11%, 83.16% and 76.00% of accumulative variation of the original variables in cotton, respectively.

The analysis displayed that the PC1 contributed to 70.72% (G.85 x TNB) and 79.67% (G.86 x Suvin) of the total variability with generations P1, P2 and BC1 in the cross G.85 x TNB and with generations P1 and F1 generations in the cross G.86 x Suvin. As for the PC2 were explained 15.57% and 12.7% of the total variation with generations P1, P2 and BC2 in the cross G.85 x TNB as well as with generations F1, F2 and BC1 in the cross G.86 x Suvin, respectively. The first six PCs contributed 69.30% of cumulative variability among 230 F2 segregants in cotton [42]. Selection during generations that have high PC1 and PC2 for studied traits are suitable and effective in the two crosses. In practice, the choice of F2 vs. backcross based populations in "second cycle" breeding is complicated by the fact that the breeder regards not only a single trait but several characters simultaneously [43].

![Biplot diagram based on first two principal component axes of six generations according to mean measured of studied traits in two crosses](image-url)
were related to yield and yield components in... Gunasekaran [41] and Nandhini et al explained that future breeding program. Similar results were in both crosses. Hence, emphasis should be would result in an increasing cotton yield means that selection based on these traits significantly correlated with number of bolls/plant and seed index traits in the cross G.86 x TNB, the trait of lint percentage and number of bolls/plant traits with the BC1 population. Also, these traits were located near the F1 population, also these traits were included near the F2 and BC1 populations in the second quarter. Whilst, the fourth quarter was include of other traits with P1 population. On the other hand, the other generations were located away from the all studied traits. The biplot analysis of the relationship between the six generations revealed that the most appropriate generations for selecting these traits were BC1 in the cross G.85 x TNB and F2 and BC1 in the cross G.86 x Suvin. Meichinger [43] declared that F2, BC1 and BC2 offer equal alternatives with respect to time, work, inbreeding level, and the amount of genetic variation released within lines in subsequent selfing generations if linkage and epistasis are of small importance. Therefore, the choice to separate a population can be based on the characteristics of the first segregating generations. The figure 1 shows that there is a divergence among the six populations, thus these diversity can be used to improve the yield and its components in cotton. The extent of variation in each trait between genotypes showed greater divergence and most traits contributed to more variance [42], thus these traits could be considered essential for selection in large populations as well as which may be

### Table 10. Results of principal component analysis for six populations based on the studied traits during the two crosses

| Traits and combination | G.85 x TNB | G.86 x Suvin |
|------------------------|------------|-------------|
|                        | PC1        | PC2         | PC3        | PC4        | PC5        | PC1        | PC2         | PC3        | PC4        | PC5        |
| P1                     | 0.12       | 0.56       | 0.25      | -0.98     | 0.00      | 0.59       | -1.24      | 0.24       | 0.15       | 0.31       |
| P2                     | 0.06       | 1.25       | -0.94     | 0.31      | -0.06     | -1.09      | -0.27      | -1.10      | -0.02      | -0.05      |
| F1                     | 3.59       | -0.03      | 0.36      | 0.27      | 0.07      | 4.21       | 0.40       | -0.04      | -0.05      | -0.11      |
| F2                     | -1.39      | -0.98      | -0.86     | -0.06     | 0.10      | -1.20      | 0.97       | 0.19       | 0.44       | -0.02      |
| BC1                    | 0.15       | -1.27      | 0.21      | 0.06      | -0.13     | -1.19      | 0.81       | 0.21       | -0.39      | 0.23       |
| BC2                    | -2.53      | 0.47       | 0.98      | 0.39      | 0.03      | -1.33      | -0.67      | 0.51       | -0.13      | -0.36      |
| Eigen value            | 4.24       | 0.93       | 0.56      | 0.26      | 0.01      | 4.78       | 0.76       | 0.32       | 0.08       | 0.06       |
| Explained variance     | 70.72      | 15.57      | 9.33      | 4.26      | 0.12      | 79.67      | 12.7       | 5.39       | 1.29       | 0.95       |
| Cumulative variance    | 70.72      | 86.29      | 95.62     | 99.88     | 100.00    | 79.67      | 92.36      | 97.76      | 99.05      | 100.00     |

The similarities and dissimilarities relationships among different six generations and studied traits in the two studied crosses are graphically displayed in a biplot of PC1 and PC2 (Fig. 3). The significance indicates that the angle between the two variables was well below 90 degrees or acute angle [44]. According to this biplot and based on the all populations, the two crosses performances displayed positive correlation for all studied traits, but, they differed in their degree of significance/insignificant and consistency in quantity. The seed cotton yield/plant trait had significant correlation with number of bolls/plant trait in the cross G.85 x TNB and with lint cotton yield/plant, lint percentage and number of bolls/plant traits in the cross G.86 x Suvin. While, boll weight trait had significantly correlated with lint cotton yield/plant and number of bolls/plant traits in the cross G.85 x TNB and with seed cotton yield/plant, lint cotton yield/plant and seed index traits in the cross G.86 x Suvin. As for lint cotton yield/plant was significant correlation with lint percentage and number of bolls/plant in the cross G.85 x TNB and with number of bolls/plant and seed index traits in the cross G.86 x Suvin. In the cross G.85 x TNB, the trait of lint percentage had significantly correlated with number of bolls/plant and seed index traits. These results means that selection based on these traits would result in an increasing cotton yield in both crosses. Hence, emphasis should be placed on such traits to improve the cotton in the future breeding program. Similar results were reported by Isong et al. [38], Vinodhana and Gunasekaran [41] and Nandhini et al. [42] who explained that highest variability by the first PCs were related to yield and yield components in cotton.
useful for an effective program to cotton breeding.

4. CONCLUSIONS

Significant divergences between the six populations for most studied traits in the two crosses were found by ANOVA. The F1 mean performance was higher than the other generations for all the studied traits in the two crosses. The ranks method and cluster analysis indicated the presence of differences among BSH methods and genes number equations for studied traits in the two crosses. The first two PCs explained the high level of accumulative variation due to the studied traits in the six populations. The principal component analysis of the relationship between the six generations revealed that the most appropriate generations for selecting these traits was BC1 in the two crosses. Backcrossing may be done for 2–5 cycles (BC2 – BC5) at Suvin parent for improving cotton in Egypt.

CONSENT

It is not applicable.

ETHICAL APPROVALS

We conducted our research after obtaining proper IEC approval.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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