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# Response of Cotton Genotypes to Water-deficit Stress using Drought Tolerance Indices and Principal Component Analysis

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***Authors' contributions***

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

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## ABSTRACT

Drought impacts on cotton cultivation and production are expected to worsen as a result of global warming and water-deficit stress. Drought tolerance indices and PCA analysis were used to evaluate drought stress responses in eleven cotton genotypes and fifteen indices' ability to identify drought-tolerant genotypes under normal and drought circumstances. Seed cotton yield (Kentar feddan$^{-1}$) was significantly affected by genotypes, years, and their interaction ($p \leq 0.05$ or 0.01) under normal and water-deficit stress conditions, according to a combined ANOVA. Except for error variance, all genetic parameters studied for seed cotton yield were higher in normal irrigation conditions than in water-deficit stress conditions. According to PCA analysis, The STI, MP, GMP,

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HM, ATI, SSPI, and TOL are suitable indicators and were similar in their ability to screen, rank and detect tolerant genotypes, due to positive correlations among each other and also the highest association with seed cotton yield in both irrigation conditions. The genotypes G4, G9, and G10 (Group A) seemed to be the most drought-tolerant and cotton productive based on mean performance, GxY heatmap analysis, drought tolerance indices, and PCA analysis. The results of our study's drought tolerance indices and PCA could be useful and appropriate for studying drought tolerance mechanisms and cotton yield improvement in Egypt.

*Keywords: Cotton; GY interaction; drought stress indices; PCA.*

## ABBREVIATIONS

*G : Genotypes*
*Y : Years*
*GY : Genotypes x years interaction*
*CV% : coefficient of variation*
*SSI : Stress susceptibility index*
*RDI : Relative Drought Index*
*TOL : Stress tolerance index*
*MP : Mean productivity index*
*YSI : Yield stability index*
*HM : Harmonic mean*
*GMP : Geometric mean productivity*
*STI : Stress tolerance index*
*YI : Yield index*
*DI : Drought resistance Index*
*YR : Yield reduction ratio*
*ATI : Abiotic tolerance index*
*SSPI : Stress susceptibility percentage index*
*SNPI : Stress non-stress production index*
*GOL : Golden mean*
*PCA : Principal component analysis*

## 1. INTRODUCTION

Cotton is a major fiber crop that supplies 35% of the world's total fiber needs [1]. In March 2021/2022, the total area harvested, yield, and production of cotton in the world were 31.77 million ha, 1.36 metric tons ha$^{-1}$, and 43.35 million metric tons, respectively. While the total area harvested, yield and production of cotton were 0.10 million ha, 1.00 metric tons ha$^{-1}$, and 0.10 million metric tons in Egypt. Cotton production increased by 5.97% in the world and 53.85% in Egypt during the 2021/2022 cropping season compared to the previous year [2].

Exploring the possibilities of drought-tolerant crops is a time requirement for all terrestrial crop species, particularly in the context of climate change [3]. Drought tolerance is defined by Hall [4] as the relative yield of a genotype compared to other genotypes subjected to the same drought stress. Solis et al. [5] cleared that drought resistance is a complex phenomenon governed by multiple genes, that manifests both drought tolerance (as tissue tolerance, photosystem maintenance, and so on) and drought avoidance (as deep root, leaf rolling, and so on) traits. According to Blum [6], drought resistance is hampered by low heritability and a lack of successful selection methods. As a result, the selection of genotypes should be adapted to drought stress conditions.

When genotypes are tested in a variety of environments (locations/years), their yield performance can vary significantly [7], especially under water-stress conditions. Betran et al. [8] stated that some researchers believe in selection under normal conditions, while, Ceccarelli and Grando [9] mentioned that some believe in selection under typical drought conditions. Nonetheless, there are many researchers who chose the middle ground and believed in selection under both normal and stressful conditions [10,11]. Several drought indices have been proposed to differentiate drought-tolerant genotypes based on a mathematical relationship between yield under normal and drought conditions. Clarke et al. [12] claimed that drought tolerance indices are based on either drought resistance or drought susceptibility of genotypes.

Principal component analysis (PCA) is the best tool for identifying genotypes that are resistant and sensitive to stress when compared to linear correlation [13]. Biplot is an exploratory data visualization tool that uses a two-dimensional scatter plot to display multivariate data. Gabriel [14] was the first to propose the notion of biplot. To display the findings of cotton trials and to pick based on a mix of correlations and drought tolerance indices, PCA is required. In addition, to identifying the correlations between drought tolerance indices, several researchers have employed the PCA to examine the relationship and diversity between several cotton germplasms [1,15,16,17,18].

The present study was carried out (1) to assess the water-deficit stress responses on seed cotton yield in eleven Egyptian cotton genotypes across five consecutive growing years under normal and water-deficit stress conditions to drought tolerance by adopting genetic parameters and drought tolerance indices, (2) study the relationship between drought tolerance indices using PCA, thus (3) identifying cotton variety drought-tolerant in Egypt.

## 2. MATERIALS AND METHODS

***Genetic material and field procedure:*** Field experiments were conducted at Sakha Agriculture Research Station, Kafr El- Sheikh Governorate, Egypt, for five consecutive years (from 2016 to 2020). Eleven cotton genotypes belonging *to Gossypium barbadense* L. each year were chosen and tested under normal and water-deficit stress conditions (Table 1). Cotton Research Institute, Agriculture Research Center, Giza, Egypt, provided healthy cotton genotype seeds. The experimental design was sown by adopting a split-plot arrangement under a randomized complete block design (RCBD) with three replications each year. Irrigation treatments were allocated in the main plots (Normal and water-deficit stress conditions). Each main plot was subdivided into eleven subplots, each of which corresponded to a different cotton genotype. Each genotype was sown in the experimental plot; each plot included five rows with a four-meter-long row. Row and plant distances were kept constant at 70 and 30 cm, respectively. The plot size kept was 13 $m^2$. For normal irrigation conditions, eight irrigations (4200 $m^3$) with one at sowing and seven other irrigations with an interval of 15 days were applied at various crop growth stages. Under the water-deficit stress conditions, the plot was irrigated five times (3150 $m^3$) with one at the time of sowing, and the other four irrigations were applied with an interval of 30 days. A basin irrigation system was used in each experiment, by means of PE pipes and a volumetric counter. Even if the water-deficit stress was severe, no supplemental irrigation was provided after drainage in the drought stress experiments. The crop was sown in a one day, and all the recommended cultural practices of cotton production in the area were done as needed, under uniform field conditions to minimize environmental variations to the maximum possible extent. After removing the border effects, the plants in each plot from the three middle rows were harvested to determine seed cotton yield/plot, which was then converted to yield Kentar/Feddan.

***Climatic data:*** Table 2 displays cultivated location climatic data such as monthly average temperature (ºC), average precipitation (mm), and relative humidity (%) from April to October over five growing seasons. The highest percentage of precipitation and relative humidity, and the lowest average temperature rates during the studied period were recorded in April during the 2017 and 2020 growing seasons.

***Statistical analysis:*** The Komolgorov-Smirnov test was used to ensure that the data distribution was normal. The combined analysis of variance (ANOVA) of seed cotton yield (Kentar/Feddan) for eleven cotton genotypes (G) in five growing years (Y) and G x Y heatmap analysis were performed using the software PBSTAT. The variances components due to the main and interaction effects of two studied experimental factors were estimated with ANOVA by Searle et al. [19]. Broad sense heritability ($H^2$) estimates were calculated using the formula suggested by Fehr [20]. Drought tolerance indices based on seed cotton yield were calculated for each genotype under normal ($Y_p$) and water-deficit stress ($Y_s$) conditions using the formulas listed in Table 3. The PCA analysis was done using Origin Pro 2021 version b 9.5.0.193 computer software program.

**Table 1. List of eleven genotypes of rice used for drought tolerance assessment**

| Code | Name | Pedigree | Origin |
|---|---|---|---|
| G1 | Giza 89 | Giza 89 x 6022 | Egypt |
| G2 | Giza 85 | Giza 67 x CB58 | Egypt |
| G3 | Giza 75 | Unknown | Egypt |
| G4 | Giza 94 | 10229 x Giza 86 | Egypt |
| G5 | Giza 89 x Giza 86 | Unknown | Egypt |
| G6 | Giza 45 | Giza 28 x Giza 7 | Egypt |
| G7 | Giza 93 | Giza 77 x S106 | Egypt |
| G8 | Giza 70 | Giza 59A x Giza 51B | Egypt |
| G9 | Giza 96 | (Giza 84 x (Giza 70 x Giza 51B)) x S62 | Egypt |
| G10 | Giza 86 | Giza 75 x Giza 81 | Egypt |
| G11 | Giza 95 | (Giza 83 x (Giza 75 x 5844)) x Giza 80 | Egypt |

**Table 2. Monthly climate data from the experimental period (April to October) in the experimental location over a five-year period**

| Climate | Years | Months | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **April** | **May** | **June** | **July** | **August** | **September** | **October** | **Mean** |
| Temperature average | 2016 | 23.85 | 25.51 | 30.01 | 29.89 | 29.76 | 28.5 | 25.47 | 27.57 |
| | 2017 | 20.22 | 28.92 | 30.86 | 30.24 | 28.1 | 23.69 | 27.12 | 27.02 |
| | 2018 | 22.69 | 26.98 | 29.01 | 30.22 | 30.08 | 28.88 | 25.53 | 27.63 |
| | 2019 | 19.86 | 26.52 | 29.27 | 30.29 | 30.48 | 28.12 | 26.08 | 27.23 |
| | 2020 | 19.58 | 24.06 | 27.69 | 29.86 | 30.44 | 30.18 | 27.12 | 26.99 |
| Average precipitation | 2016 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.48 | 0.08 |
| | 2017 | 2.68 | 0.36 | 0.34 | 0.00 | 0.00 | 1.38 | 0.07 | 0.69 |
| | 2018 | 0.07 | 0.00 | 0.00 | 0.1 | 0.00 | 0.00 | 0.15 | 0.05 |
| | 2019 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.55 | 0.10 |
| | 2020 | 3.43 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.07 | 0.50 |
| Relative humidity | 2016 | 50.19 | 48.32 | 48.66 | 54.51 | 57.41 | 55.93 | 63.42 | 54.06 |
| | 2017 | 59.74 | 50.23 | 53.55 | 55.35 | 57.56 | 63.00 | 60.19 | 57.09 |
| | 2018 | 52.38 | 51.39 | 48.67 | 54.97 | 57.37 | 57.23 | 58.85 | 54.41 |
| | 2019 | 56.79 | 44.73 | 52.85 | 52.9 | 55.16 | 58.00 | 62.09 | 54.64 |
| | 2020 | 64.05 | 59.35 | 51.10 | 54.99 | 56.65 | 58.92 | 60.19 | 57.89 |

*Source: Climate Change Information Center and Renewable Energy, Agriculture Research Center, Cairo, Egypt.*

**Table 3. Drought tolerance indices used for the evaluation of rice genotypes to water-deficit stress conditions**

| No. | Drought tolerance indices | Equation | Reference |
|---|---|---|---|
| 1 | Stress susceptibility index (SSI) | $[1-(Y_s/Y_p)]/[1-(\bar{Y}_s/\bar{Y}_p)]$ | Fischer and Maurer [21] |
| 2 | Relative Drought Index (RDI) | $(Y_s/Y_p)/(\bar{Y}_s/\bar{Y}_p)$ | |
| 3 | Stress tolerance index (TOL) | $\mathrm{Y_p} - \mathrm{Y_s}$ | Rosielle and Hamblin [22] |
| 4 | Mean productivity index (MP) | $(Y_p + Y_s)/2$ | |
| 5 | Yield stability index (YSI) | $Y_s/Y_p$ | Bouslama and Schapaugh [23] |
| 6 | Harmonic mean (HM) | $\left[2\left(Y_p x Y_s\right)\right]/(Y_p + Y_s)$ | Hossain et al. [24] |
| 7 | Geometric mean productivity (GMP) | $(\mathrm{Y_p x Y_s})^{1/2}$ | Fernandez [11] |
| 8 | Stress tolerance index (STI) | $(Y_p x Y_s)/(\bar{Y}_p)^2$ | |
| 9 | Yield index (YI) | $Y_s/\bar{Y}_s$ | Gavuzzi et al. [25] |
| 10 | Drought resistance Index (DI) | $[Y_s x(Y_s/Y_p)]/\bar{Y}_s$ | Lan [26] |
| 11 | Yield reduction ratio (YR) | $1-(Y_S/Y_p)$ | Golestani–Araghi and Assad [27] |
| 12 | Abiotic tolerance index (ATI) | $\left[(Y_p - Y_s)/(\bar{Y}_p - \bar{Y}_s)\right]x\left[\sqrt{Y_p x Y_s}\right]$ | Moosavi et al. [28] |
| 13 | Stress susceptibility percentage index (SSPI) | $\left[(Y_p - Y_s)/2(\bar{Y}_p)\right]x100$ | |
| 14 | Stress non-stress production index (SNPI) | $\left[\sqrt[3]{(Y_p + Y_s)/(Y_p - Y_s)}\right]x\left[\sqrt[3]{Y_p x Y_s x Y_s}\right]$ | |
| 15 | Golden mean (GOL) | $(Y_p + Y_s)/(Y_p - Y_s)$ | Moradi et al. [29] |

*$\boldsymbol{Y_p}$ and $\boldsymbol{Y_s}$: grain yield of each genotype under non-stress and stress conditions, respectively; $\boldsymbol{\bar{Y}_p}$and $\boldsymbol{\bar{Y}_s}$: mean grain yield of all genotypes in non-stress and stress conditions, respectively*

## 3. RESULTS

We studied fifteen drought tolerance-related indicators of seed cotton output under drought stress conditions for five consecutive years (from 2016 to 2020) in order to analyze the impacts of drought stress on eleven Egyptian cotton materials.

***Combined ANOVA and genetic parameters:*** The data of combined ANOVA and genetic parameters for each trial individually for seed cotton yield (Kentar/Feddan) is presented in Table 4. The combined ANOVA table showed that seed cotton yield was significantly affected ($p \leq 0.05$ or 0.01) by genotype (G), years (Y), and GY interaction in both irrigation conditions. The effects of E, G, and GY interaction collectively explained 83.94% and 74.45 % of the total cotton yield variation under normal irrigation and water-deficit stress conditions, respectively. The G (39.01%) explained most of the total SS, followed by the GY interaction (35.33%) under normal irrigation conditions, while the opposite was true for water-deficit stress conditions (21.40% and 45.83%, respectively). In normal irrigation and water-deficit stress conditions, seed cotton yield displayed low and moderate coefficient of variation (CV%) values of 8.45% and 14.78%, respectively. According to ANOVA analysis, which assumes a random-effects model, all genetic parameters calculated for seed cotton yield were higher in normal irrigation conditions compared with water-deficit stress conditions, except for error variance. The variance due to GY interaction was greater than the other variances in both irrigation conditions. The values of $H^2$ were high ($H^2$>0.60) and moderate (0.30<$H^2$<0.60) for seed cotton yield under normal irrigation and water-deficit stress conditions, respectively (Table 4).

***Mean Performance and GY heatmap analysis:*** Mean seed cotton yield comparisons in both irrigation conditions showed significant differences among evaluated genotypes in each growing year. Over the five years studied, normal irrigation conditions resulted in a significant increase in seed cotton yield when compared to water-deficit stress conditions (Fig.1). The average environmental seed cotton yield of genotypes ranged from 7.94 (G7 in 2019) to 16.33 (G4 in 2018) and from 4.72 (G7 in 2018) to 12.61 (G1 in 2020) under normal irrigation and water-deficit stress conditions, respectively. Based on the mean of all investigated genotypes, the growing years 2018 (13.35) and 2019 (8.81) had the highest seed cotton yield compared with other years under normal irrigation and water-deficit stress conditions, respectively.

GY heatmap analysis of seed cotton yield was used to create a visual comparison of the effects of the growing years on the genotypes in both irrigation conditions, as well as to determine the range of water-deficit stress responses detectable in these genotypes (Fig. 1). The GY heatmap analysis of both irrigation conditions revealed two dendrograms: the five years on top, and that influenced the distribution of eleven cotton genotypes on the left. In both irrigation conditions, the top dendrogram classified the growing years into two distinct clusters. The first cluster included the 2019 and 2020 years, as well as the 2020 year under normal irrigation and water-deficit stress conditions. The second cluster included the remaining years in both irrigation conditions. As for the left dendrogram, eleven genotypes could be classified for five and seven clusters in normal irrigation and water-deficit stress conditions, respectively. Genotypes within the cluster have the least variance and genetic distance, whereas genotypes between clusters differ and have the greatest genetic distance.

The G4 genotype in the second cluster gave the best seed cotton yield in most growing years, followed by the genotypes in the fifth cluster (G2, G9, and G10) under normal irrigation conditions. The genotype G7 in the third cluster had the best cotton yield in 2016, 2017, and 2018 years under normal irrigation conditions. Based on the heat map under water-deficit stress conditions, the G3 and G4 genotypes in the sixth and fifth clusters, respectively, were among the best performers of cotton yield across most growing years, followed by the genotypes in the second cluster (G9 and G10). The G1and G2 genotypes in the fourth and seventh clusters recorded the highest seed cotton yield in the 2020 and 2017 years, respectively, and moderate to low cotton yield in the other years. In contrast, the other genotypes in the other clusters were intermediate or low in GY interactions in both irrigation conditions. Generally, the G8 and G6 genotypes recorded the lowest seed cotton yield in normal irrigation and water-deficit stress conditions, respectively.

**Table 4. Combined ANOVA and genetic parameters across five years for seed cotton yield of 24 genotypes under normal irrigation and water-deficit stress conditions**

| Source of Variation | df | Normal irrigation conditions | | | Water-deficit stress conditions | | |
|---|---|---|---|---|---|---|---|
| | | Sums of Squares (SS) | Mean of Squares | SS% | Sums of Squares (SS) | Mean of Squares | SS% |
| Years (Y) | 4 | 87.25 | 21.81** | 9.60 | 43.93 | 10.98** | 7.23 |
| Replication/Y | 10 | 34.38 | 3.44** | 3.78 | 13.91 | 1.39ns | 2.29 |
| Genotype (G) | 10 | 354.43 | 35.44** | 39.01 | 130.07 | 13.01* | 21.40 |
| G x Y | 40 | 321.00 | 8.03** | 35.33 | 278.60 | 6.97** | 45.83 |
| Error | 100 | 111.51 | 1.12 | 12.27 | 141.38 | 1.41 | 23.26 |
| CV% | | 8.45 | | | 14.78 | | |
| **Genetic Parameters** | | | | | | | |
| $V_G$ | | 1.83 | | | 0.40 | | |
| $V_{GY}$ | | 2.30 | | | 1.85 | | |
| $V_E$ | | 1.12 | | | 1.41 | | |
| $V_{Ph}$ Mean | | 2.36 | | | 0.87 | | |
| $H^2$ Mean | | 77.36 | | | 46.45 | | |

*$V_G$: genotypic variance; $V_{GY}$: genotype x year interaction variance; $V_E$: error variance; $V_{Ph}$ mean: phenotypic variance on entry-mean basis; $H^2$ mean: broad-sense heritability on entry-mean basis (%). Statistically significant differences at *p ≤ 0.05 and **p ≤ 0.01; ns: indicate the non-significant difference.*

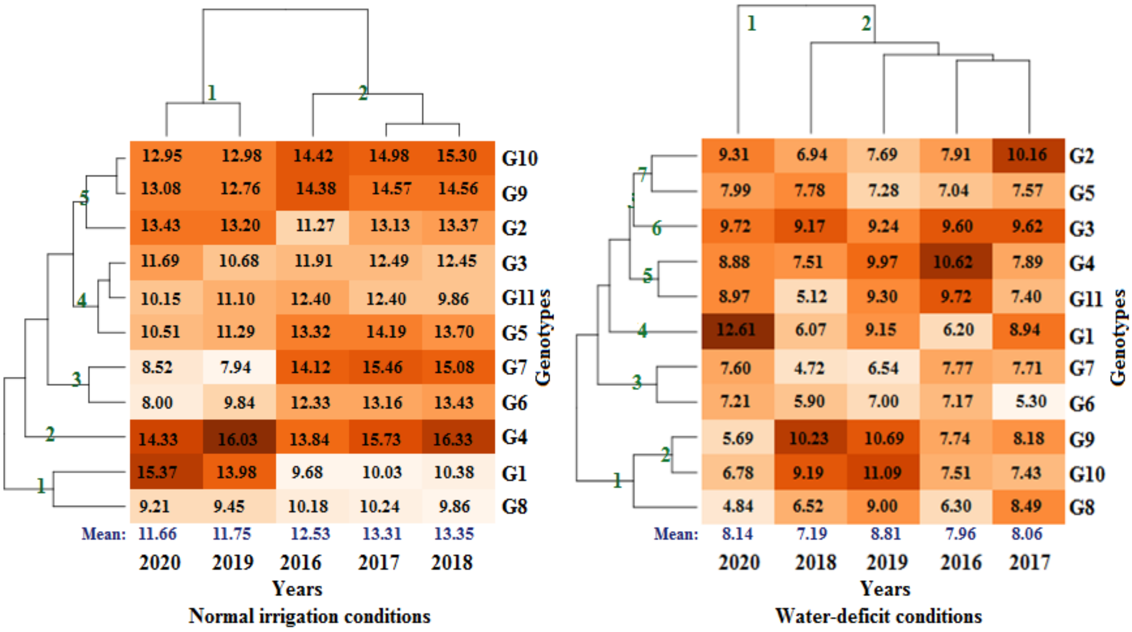


**Fig. 1. Cluster heat map analysis of classified genotypes in growing years during normal irrigation and water-deficit stress conditions. The genotypes key names can be found in Table 1**

**Drought Tolerance Indices:** Fifteen drought tolerance indices based on seed cotton yield potential and response were calculated, to assess the drought tolerance of eleven cotton genotypes under normal irrigation (Yp) and water-deficit stress (Ys) conditions (Table 5). The low values of the SSI, TOL, YR, ATI, and SSPI indices indicate that the genotypes are low sensitive to water stress. In comparison, the high values of the MP, GMP, STI, YI, YSI, DI, SNPI, RDI, HM, and GOL indices indicate that the genotypes are drought-tolerant. The investigated genotypes showed significant differences in seed cotton yield under normal irrigation and water-deficit stress conditions. Over five growing years, the seed cotton yield of eleven genotypes decreased under water-deficit stress compared to normal irrigation conditions. Seed cotton yield ranged from 9.79 Kentar/Feddan (G8) to 15.45 Kentar/Feddan (G4) under Yp conditions, and from 6.52 Kentar/Feddan (G6) to 9.47 Kentar/Feddan (G3) under Ys conditions.

Lower SSI, TOL, YR, ATI, and SSPI values, as well as higher YSI, RDI, and GOL values were recorded by the genotypes G1, G3, and G8. As a result, these genotypes were identified as the most drought-resistant and desirable under Ys based on these indices. The YI, DI, and SNPI indices were high in G1, and G3 during the Ys, and G4 during Yp. However, the genotypes G6, and G7 by the indices of SSI, YR (high), Yi, YSI, DI, SNPI, RDI, and GOL (low) , and the genotypes G4 and G10 by TOL, ATI, and SSPI indices (high) were identified as drought-susceptible.

The genotypes G4, G9, and G10 exhibited the highest values by MP, GMP, STI, and HM indices with high productivity under Yp, and moderate-to-high productivity under Ys. Therefore, these genotypes were classified as drought tolerant in both irrigation conditions. Opposite, the genotypes G6, G7, and G8 showed low MP, GMP, STI, and HM values with low productivity in both Yp, and Ys, but the G7 genotype had moderate productivity in Yp. As a result, these findings suggest that these genotypes are more sensitive to drought. With the exception of the previously identified sensitive and tolerant genotypes, all drought tolerance indices in this study classified the remaining genotypes as semi-tolerant or semi-sensitive to drought stress.

**Table 5. Comparison of drought indices for eleven cotton genotypes based on seed cotton yield (Kentar/Feddan) under normal irrigation (Yp) and water-deficit stress (Ys) conditions (averaged over five years)**

| Genotypes | Drought Tolerance Indices | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Yp** | **Ys** | **SSI** | **TOL** | **YR** | **ATI** | **SSPI** | **MP** | **GMP** | **STI** | **YI** | **YSI** | **DI** | **SNPI** | **RDI** | **HM** | **GOL** |
| G1 | 11.88 | 8.59 | 0.77 | 3.29 | 0.28 | 7.41 | 13.14 | 10.24 | 10.10 | 0.65 | 1.07 | 0.72 | 0.77 | 17.60 | 1.13 | 9.97 | 6.22 |
| G2 | 12.88 | 8.4 | 0.97 | 4.48 | 0.35 | 10.40 | 17.89 | 10.64 | 10.40 | 0.69 | 1.05 | 0.65 | 0.68 | 16.28 | 1.02 | 10.17 | 4.75 |
| G3 | 11.84 | 9.47 | 0.56 | 2.37 | 0.20 | 5.60 | 9.47 | 10.66 | 10.59 | 0.72 | 1.18 | 0.80 | 0.94 | 21.21 | 1.25 | 10.52 | 8.99 |
| G4 | 15.45 | 8.97 | 1.17 | 6.48 | 0.42 | 17.02 | 25.88 | 12.21 | 11.77 | 0.88 | 1.12 | 0.58 | 0.65 | 16.73 | 0.90 | 11.35 | 3.77 |
| G5 | 12.6 | 7.53 | 1.12 | 5.07 | 0.40 | 11.02 | 20.25 | 10.07 | 9.74 | 0.61 | 0.94 | 0.60 | 0.56 | 14.16 | 0.93 | 9.43 | 3.97 |
| G6 | 11.35 | 6.52 | 1.19 | 4.83 | 0.43 | 9.27 | 19.29 | 8.94 | 8.60 | 0.47 | 0.81 | 0.57 | 0.47 | 12.13 | 0.89 | 8.28 | 3.70 |
| G7 | 12.22 | 6.87 | 1.22 | 5.35 | 0.44 | 10.94 | 21.37 | 9.55 | 9.16 | 0.54 | 0.85 | 0.56 | 0.48 | 12.72 | 0.88 | 8.80 | 3.57 |
| G8 | 9.79 | 7.03 | 0.79 | 2.76 | 0.28 | 5.11 | 11.02 | 8.41 | 8.30 | 0.44 | 0.87 | 0.72 | 0.63 | 14.34 | 1.12 | 8.18 | 6.09 |
| G9 | 13.87 | 8.51 | 1.08 | 5.36 | 0.39 | 12.99 | 21.41 | 11.19 | 10.86 | 0.75 | 1.06 | 0.61 | 0.65 | 16.13 | 0.96 | 10.55 | 4.18 |
| G10 | 14.13 | 8.4 | 1.13 | 5.73 | 0.41 | 13.93 | 22.89 | 11.27 | 10.89 | 0.76 | 1.05 | 0.59 | 0.62 | 15.77 | 0.93 | 10.54 | 3.93 |
| G11 | 11.69 | 8.1 | 0.86 | 3.59 | 0.31 | 7.79 | 14.34 | 9.90 | 9.73 | 0.60 | 1.01 | 0.69 | 0.70 | 16.17 | 1.08 | 9.57 | 5.51 |
| Minimum | 9.79 | 6.52 | 0.56 | 2.37 | 0.20 | 5.11 | 9.47 | 8.41 | 8.30 | 0.44 | 0.81 | 0.56 | 0.47 | 12.13 | 0.88 | 8.18 | 3.57 |
| Maximum | 15.45 | 9.47 | 1.22 | 6.48 | 0.44 | 17.02 | 25.88 | 12.21 | 11.77 | 0.88 | 1.18 | 0.80 | 0.94 | 21.21 | 1.25 | 11.35 | 8.99 |
| Mean | 12.52 | 8.04 | 0.99 | 4.48 | 0.36 | 10.13 | 17.90 | 10.28 | 10.01 | 0.65 | 1.00 | 0.64 | 0.65 | 15.75 | 1.01 | 9.76 | 4.97 |

*The genotypes and drought tolerance indices key names can be found in Tables 1 and 3, respectively*

**Principal component analysis:** Principal component analysis (PCA) was used to identify drought-tolerant and sensitive genotypes, as well as to gain a clear understanding of the relationships between drought tolerance indices in both irrigation conditions. Out of all PCs, the two first main PCs (PC1 and PC2) were kept for the final analysis because they both have eigenvalues greater than one and explain 99.50% of the total variance of all analyzed variables. The PC1 explains 54.67% of the total variance of variables and is highly positively correlated with indices of SSI, YR, TOL, SSPI, and ATI, and positively correlated with indices of MP, GMP, and STI under Yp (Fig. 2A). While, the PC2 accounted for 44.83% of the total variation of analyzed variables and strongly positively correlated with indices of STI, MP, GMP, HM, and YI, and positively correlated with other indices under Yp, and Ys, except for SSI, and YR (Fig. 2B). Generally, PC1 and PC2 are positively correlated with STI, MP, GMP, HM, and YI indices in both irrigation conditions.

A perfect positive correlation had observed between YS and YI, between SSI and YR, between TOL and SSPI, between GMP and STI, as well as between YSI and RDI, because the angles between them are zero (Fig. 3). Our findings revealed that most drought indices had below 90-degree angles (sharp angled), indicating a positive correlation between these variables. A high and positive correlation (smallest sharp angles) was recorded among Yp with TOL, ATI, SSPI, MP, GMP, STI, YI, and HM indices, as well as among Ys with MP, GMP, STI, YI, DI, SNPI, and HM indices. A strong positive association was observed among SSI, TOL, YR, SSPI, and ATI indices, among MP, GMP, STI, YI, SNPI, and HM indices, among YSI, DI, SNPI, RDI, and GOL indices, and among YI, DI, SNPI, and HM indices, suggesting that these indices are closely associated in the ranking of the genotypes. ATI had highly positively correlated with MP, GMP, STI, and HM indices. The other relationships between the drought tolerance indices were positive (low) or negative, depending on whether the angles between them were acute (large) or obtuse, respectively (Fig. 3).

As shown in Fig. 3, the PCA analysis for seed cotton yield and drought tolerance indicators also allowed cotton genotypes to be divided into four groups based on their phenotypic similarities under normal irrigation and water-deficit stress conditions. The first quarter (the first group) was occupied by the genotypes G4, G9, and G10 using STI, MP, GMP, HM, ATI, SSPI, and TOL, which showed the highest PC1 and PC2 as well as the highest and moderate seed cotton yield in Yp and Ys, respectively. The second group comprised genotypes G5, G6 and G7 using SSI and YR, which were located in the fourth quarter (the highest PC1 and the lowest PC2) and showed medium cotton yield in Yp. The genotypes G8, and G11, which were discovered in the third quarter, formed the third group (the lowest PC1, and PC2), and had low to moderate grain yield performance in both conditions, and associated with YSI, and RDI. The genotypes G1, G2, and G3 by YI, SNPI, DI, GOL, RDI, and YSI had the lowest PC1 and the greatest PC2 in the fourth group (the second quarter), which exhibited a high and moderate yield response in Ys and Yp, respectively.

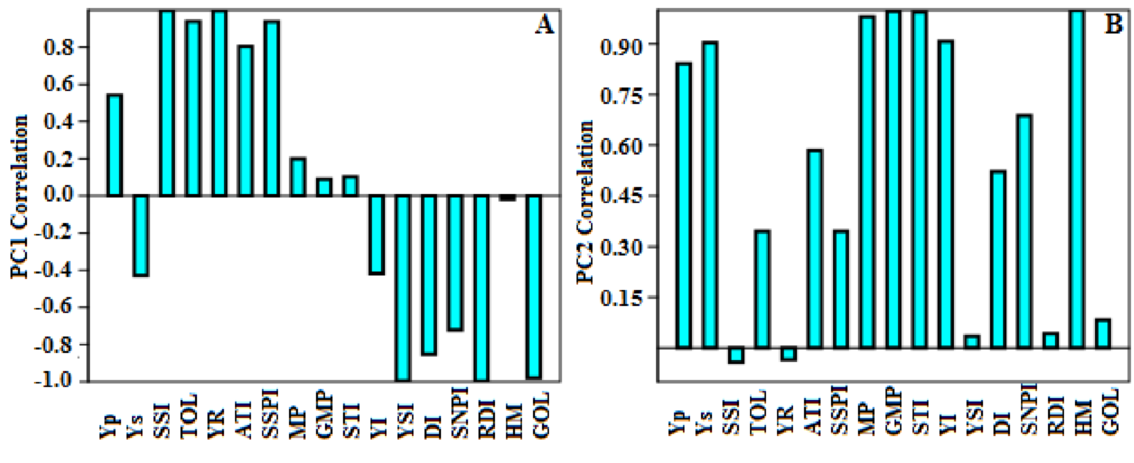


**Fig. 2. The correlation of PC1 (A) and PC2 (B) with drought tolerance indices based on the variables analyzed. The drought tolerance indices key names can be found in Table 3**

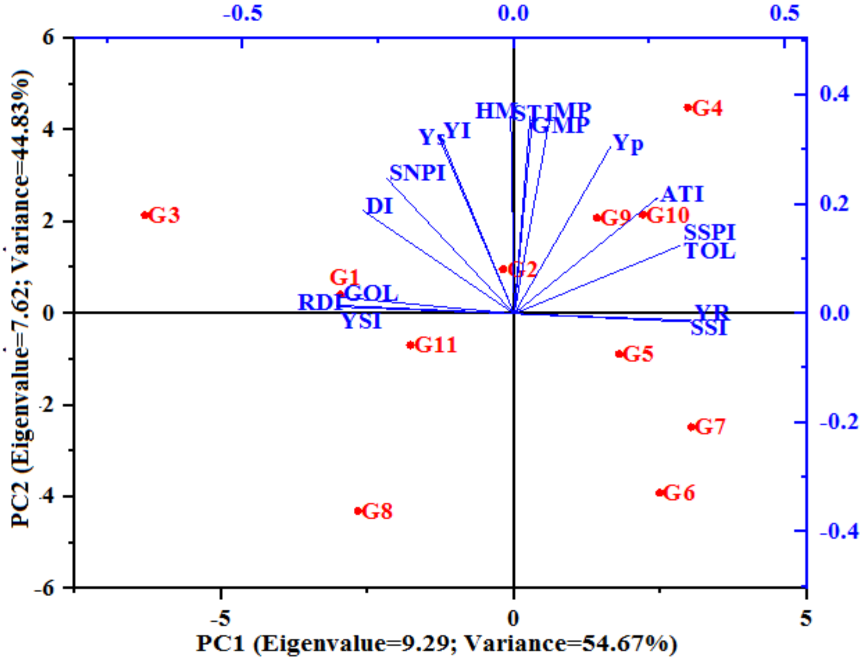


**Fig 3. Biplot diagram based on PC1 and PC2 shows similarities and dissimilarities relationships among the drought indices for eleven cotton genotypes based on seed cotton yield under normal irrigation (Yp) and water-deficit stress (Ys) conditions. The genotypes and drought tolerance indices key names can be found in Tables 1 and 3, respectively**

## 4. DISCUSSION

Cotton genotypes tolerant to drought stress and sandy soil conditions are especially needed in areas where drought is a major stress factor affecting cotton agriculture [16]. Results of combined ANOVA exhibited statistically significant differences ($p \leq 0.05$ or 0.01) among genotypes (G), years (Y), and GY interaction for seed cotton yield under normal irrigation and water-deficit stress conditions. The results of the three-year ANOVA revealed that following water treatment, seed cotton yield exhibited significant differences [15]. The SS% of GY interaction and experimental error was higher in water-deficit stress conditions than in normal irrigation conditions, opposite for SS% of other sources. These findings show that genotypes have a lot of variation and are distinct, allowing us to choose genotypes under water-deficit stress conditions. In both irrigated and rainfed trials, Ayele et al. [30] and Abo Sen et al. [31] found high variability in yield among cotton genotypes. Our findings also revealed the variability and inconsistency in seed cotton yield responses among investigated genotypes in both irrigation conditions over a five-year period. In the current study, the results of ANOVA are congruent with previous findings on cotton genotypes under normal irrigation and water-deficit stress conditions by other researchers [32, 33, 34, 1, and 35].

Water-deficit stress conditions exhibited a higher CV% than normal irrigation conditions, indicating that water-deficit stress generated substantial variability in seed cotton yield among examined cotton genotypes. Under water-deficit stress, high CV% values imply that the cotton genotypes chosen are plentiful, the drought effect is visible, and the results are representative [15]. The low CV% showed the accuracy of the cotton experiment under dry irrigation conditions, according to Manan et al., [36].

Learning about the inheritance pattern of targeted traits in cotton, including variance components and degree of heritability, is critical for developing a breeding plan to improve drought stress tolerance in the targeted genotypes [1]. Generally, breeding for drought tolerance is very complicated. In this study, the heritable part of the total observed variability has been studied by variance components and the degree of heritability, which indicate the genetic and non-genetic factors (GY interaction) may be played an important role in the manifestation of seed cotton yield. Similar to Mahmood et al. [1], the inheritance of seed cotton yield was comparatively low in water-deficit stress conditions than in normal irrigation conditions. The results indicate the importance of seed cotton yield in providing a high amount of genetic gain, which leads to the evaluation and selection of superior genotypes under water-deficit stress conditions, as reported by El-Hashash and Agwa [3].

In both irrigation conditions, GY heatmap analysis of seed cotton yield categorized genotypes and years into different independent clusters. According to the heatmap, the genotypes G4, G9, and G10 in both irrigation conditions, G2 and G3 in normal irrigation and water-deficit stress conditions, respectively, had the highest cotton yield across most years studied, while the genotypes G8 and G6 had the lowest cotton yield under normal irrigation and water-deficit stress conditions, respectively. Ayele et al. [30], Yehia [34], and Eid et al. [16] observed the same trend when compared to water-deficit stress conditions; cotton genotypes in normal irrigation conditions showed higher seed cotton yield, which ranged from 11.12% by G3 to 28.03% by G7, suggesting genetic variability in eleven studied genotypes for drought tolerance. Also, Grzesiak et al. [13] and Sun et al. [15] found a considerable decrease in yield in drought-sensitive genotypes, whereas yield decline was significantly lower in drought-tolerant genotypes. These genotypes produced better cotton productivity under normal irrigation conditions, but some genotypes also performed well under water-deficit stress conditions, suggesting their relative variable performance and strong sensitivity to environmental fluctuation [3]. As a result, drought tolerance indices must be used to assess seed cotton production for each of the eleven genotypes in order to find genotypes with tolerant and superior cotton yield under water-deficit stress conditions.

The genotypes G1 and G3 by SSI, TOL, YR, ATI, and SSPI (lower values), YI, YSI, DI, SNPI, RDI, and GOL (higher values) under Ys, and the genotypes G4, G9 and G10 by MP, GMP, STI, and HM (higher values) under both Yp, and Ys were shown to be the most drought-tolerant genotypes. In contrast, most drought tolerance indicators showed genotypes G6, and G7 as more sensitive to drought with low productivity under both Yp and Ys. These findings showed that the SSI, TOL, YR, ATI, SSPI, YSI, RDI, GOL, YI, DI, and SNPI, as well as the MP, GMP, STI, and HM indices, ranked and selected genotypes in a similar way. Clark et al. [10], Moosavi et al. [28], and Singh et al. [37] observed a similar pattern. Furthermore, most drought indices differed in identifying tolerant genotypes but were similar in identifying sensitive genotypes. MP, GMP, and STI indices have successfully helped to discriminate the genotypes as they revealed a minimal reduction in yield in response to a stress condition, and distinguished tolerant genotypes from sensitive genotypes [38, 34, 18]. In both irrigation conditions, the STI, MP, GMP, and HM indices were useful parameters for selecting high-yielding cotton genotypes. These findings are in agreement with those obtained by El-Hashash et al. [3] in barley, El-Hashash and EL-Agoury (2019) in rice, Shahid et al. [39] in wheat, and Ghodrat and Bahran [17] in cotton. It appears that other tolerance indices were successful in selecting genotypes with high yield under Ys but failed to select genotypes with appropriate yield in both irrigation conditions; thus, these indices are better for determining drought tolerance levels with a relative decrease in yield. Opposite, Clarke et al. [10] suggested that genotypes with a considerable loss in yield might have a higher-yielding capability in both conditions.

Because PCA analysis was a more effective method of identifying stress-resistant and sensitive genotypes [13], we employed statistical analysis PCA, and also to evaluate the correlations among the drought tolerance indices. Only the PC1 and PC2 extracted PCs had eigenvalues higher than one and explained 54.67% and 44.83%, respectively, and collectively they contributed 99.50% of the total variation for variables during normal irrigation and water-deficit stress conditions. Therefore, they can be used as the basis for assessing genotypes and drought tolerance indices. Generally, PC1 and PC2 are highly or lowly positively correlated with most drought tolerance indices in both irrigation conditions. These findings are consistent with those of other cotton studies (such as [40,17,18].

The indices of YS and YI; SSI and YR; TOL and SSPI; GMP and STI; and YSI and RDI are similar in the ranking of genotypes for drought tolerance, due to a complete positive correlation between them. Positive correlations among most drought indices were found, but they differed in their degree and consistency in quantity and significance. In general, the indices of MP, GMP, STI, YI, and HM were highly positively correlated with seed cotton yield in both Yp and Ys, indicating that they rank genotypes in a similar fashion in these indices and that selection based on these indices will result in increased seed cotton yield in both conditions. Earlier studies on cotton found similar positive associations among drought tolerance indices (for example, [40, 34, 17, 41]).

The investigated genotypes were grouped into four categories based on their performance and drought tolerance indices using biplot analyses, according to Fernandez's (1992) classification. G4, G9, and G10 genotypes with STI, MP, GMP, HM, ATI, SSPI, and TOL showed high yield in both Yp and Ys (group A). Group B comprised the genotypes G5, G6, and G7 using SSI and YR, which had a high yield response in Yp. Under Ys, the genotypes G1, G2, and G3 employing YI, SNPI, DI, GOL, RDI, and YSI produced good yields and were placed in Group C. G8, and G11 genotypes have low grain yield performance in both Yp and Ys based on most evaluated indices (Group D). Based on our results, The STI, MP, GMP, HM, ATI, SSPI, and TOL indices are the best indicators for identifying drought-tolerant genotypes. These previous results have been reported in several studies on cotton, such as Yehia [34], Ghodrat and Bahran [17] and Quevedo et al. [41]. Also, the genotypes G4, G9, and G10 were found to be more tolerant under drought stress, and poor climatic conditions and have the potential to increase the sustainable productivity of cotton in Egypt.

## 5. CONCLUSIONS

Due to the different climatic conditions throughout five years, our findings support the existence of a wide range of genotypic variations in response to drought stress in cotton genotypes. Most drought tolerance indices showed similarity and effectiveness in ranking and detecting drought-tolerant genotypes over growing years in both irrigation conditions. STI, MP, GMP, HM, ATI, SSPI, and TOL indices and PCA analysis could be used as suitable methods for studying the drought tolerance mechanisms in cotton and were effective in identifying the G4, G9, and G10 as drought-tolerant genotypes with high yield potential. Thus, these genotypes are recommended under the water-deficit stress and poor climatic conditions in Egypt.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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