Genetic Variability of Maize Hybrids and Populations and Interrelationships among Grain Yield and Its Related Traits under Drought and Low N Using Multivariate Analysis

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ABSTRACT

One of the best biometrical methods for estimating genetic diversity among germplasm collections is multivariate analysis; it is used to study their variability and genetic relatedness in order to increase their value in plant breeding programs. The objectives of the present study were to: (i) evaluate the magnitude of genetic diversity, based on phenotypic data, among 19 maize genotypes, under drought and/or low N stressed conditions in the field, using principle component analysis (PCA) and (ii) assess the interrelationships between maize grain yield and its related traits under such stressed conditions using genotype × trait (GT) biplot analysis. An experiment was...
conducted in two seasons using a split-split plot design with 3 replications, where 2 irrigation regimes (well-watered and water stressed at flowering) occupied the main plots, three N rates (high N, medium N and low N) occupied the sub plots and 19 maize genotypes occupied the sub-sub plots. The genotypes were evaluated for 19 agronomic traits. Analysis of variance was performed under each of the six environments. Significant differences (p≤0.01) were recorded among the maize genotypes for all studied traits under each environment. The best genotypes for each trait were identified. Results of the GT biplot indicated that high means of 100-kernel weight (100-KW), ears/plant (EPP), ear height, days to silking, days to anthesis, plant height, and chlorophyll concentration index (CCI) under water stress (WS), kernels/row (KPR), EPP, 100-KW and CCI under low N and KPR, EPP and 100-KW under WS combined with low N environment and low values of anthesis-silking interval (ASI) under the three stressed environments could be considered selection criteria for high grain yield under respective stressed environments and for drought and/or low N tolerance. It is recommended to select for high values of KPR, EPP and 100-KW and low value of ASI in order to increase grain yield under such stressed conditions.

**Keywords**: Maize collections; phenotypic data; PCA; GT-biplot; selection criteria.

1. **INTRODUCTION**

Maize (*Zea mays* L.) is the second cereal crop, after wheat, in Egypt with regard to the cultivated area and production. Egypt in 2018 grew maize in 935,778 hectares producing about 7.8 million tons of grains, with an average yield of about 7.8 t ha⁻¹ [1]. It is consumed mainly as feed by poultry and as food by humans. Also, it is used as a basic raw material for several industrial products, such as oil, starch, protein, alcoholic beverages, paper industries, etc. [2]. Maize grain contains 72% starch, 10% protein, 4.80% oil, 8.50% fiber, 30% sugar and 1.70% ash [3].

In Egypt, the local production of maize crop is not sufficient to satisfy the local consumption, which is about 16 million tons, requiring importation of about 9 million tons. To improve productivity, Egypt has increased land area under maize by cultivating maize in sandy soils characterized with low in N and in water holding capacity; so it is needed to improve the maize productivity under such stresses, i.e. to improve drought and/or low-N tolerance of Egyptian maize cultivars.

To begin an efficient breeding program for improving water stress and low N tolerance of maize, germplasm collections should be assessed for productivity and agronomic performance under these stresses for the identification of the suitable sources for isolating tolerant inbred lines for use in developing tolerant hybrids to these stresses [4-6]. The availability of sufficient genetic variability would lead to the success in the development of tolerant maize hybrids to such stresses [3]. The wider the genetic diversity, the greater the chances for successful development of new tolerant maize hybrids. Assessment of the genetic diversity in the available germplasm is of high importance for effective utilization of such germplasm [7]. To obtain superior cultivars, maize breeders should use, in their breeding programs, unrelated material, select heterotic inbred lines and pools for the development of superior hybrids [8].

Two approaches (morphological and molecular) are used in assessment of genetic diversity in the available germplasm. Though morphological assessment is limited by the environmental effect of on trait performance, shows low heritability, is labor intensive, time consuming, requires large population size, and does not cover the whole genome [9], it offers an unparalleled way of identification of phenotypic variation. Morphological evaluation is the first step for the assessment, characterization and grouping of germplasm collections to increase their use value in maize breeding program [10]. Phenotypic description is the foremost step in germplasm identification and classification [11]. Phenotypic characteristics are easy to measure, inexpensive and are trustable for estimation of heritability [11-13]. For these reasons, they are highly preferable in developing countries, where labor is not costly. Phenotypic assessment was efficient for analysis of genetic diversity in maize [14-17].

Studies on genetic diversity in maize are published by various authors, therefore providing a rationale on the importance of such studies [18,19]. Dao et al. [20] reported that genetic variability among different populations provides and strengthens the adaptability to changing environments and market requirements. Genetic progress in yield and other traits of economic...
importance in any breeding program is highly dependent and influenced by the genetic variability within the breeding population; therefore, selection of the improved breeding material depends on the level of available genetic variability [19].

One of the best biometrical methods for estimating genetic diversity among available germplasm is multivariate analysis; it is used to study their variability and genetic relatedness in order to increase their value in plant breeding programs [21,22]. The principle component analysis (PCA) and cluster analysis are preferred biometrical tools for morphological assessment of genotypes and their classification on similarity basis based on this approach [23]. Multivariate analyses have been utilized for maize in many countries [24,25].

Researchers studied several traits in different environments, but usually faced problems in assessments of these traits. The problem gets complicated in selection studies especially when there is a negative interaction between the primary trait of the experiments and the other traits [26]. Therefore, genotype main effect plus genotype × environment interaction (GGE) biplot method is considered as the best method for trustable assessments in multi-environment experiments [27]. Assessments are usually performed over PC1 and PC2 axes calculated from the data of rows and columns from a two dimensional array produced by the combination of genotypes and environments in multiple environment datasets [28]. This method is also used for visual assessments of correlations among studied traits through genotype × trait (GT)-biplot graph [28].

Information on the interrelationships among grain yield and its related traits is desirable for designing appropriate breeding strategies most especially under stress conditions. Several statistical tools have been employed in the study of interrelationship among traits. The most commonly used methods by breeders include path coefficient analysis and GGE biplot. The heritability of grain yield under stress conditions is usually low [29]. This necessitates the use of secondary traits with strong association with yield for indirect selection for yield improvement under stress conditions [30]. Secondary traits such as ears per plant, stay green characteristic, and anthesis-silking interval have strong associations with yield under drought conditions and have been used to select for higher levels of tolerance to drought in maize [5,6,31-33]. Maize breeders in IITA utilize a selection index that integrates increased grain yield under drought and well-watered environments with a short anthesis-silking interval, increased ears per plant, good stay green characteristic, and good scores for plant aspect and ear aspect under drought for improvement of maize germplasm for tolerance to drought [30,34-36,67,68].

The objectives of the present study were to: (i) evaluate the magnitude of genetic diversity, based on phenotypic data, among 19 maize genotypes, under drought and/or low N stressed conditions in the field, using principle component analysis (PCA) and (ii) assess the interrelationships between maize grain yield and its related traits under such stressed conditions using genotype × trait (GT) biplot analysis. This information will be useful for identifying genotypes for broadening the genetic base in the gene pools of maize improvement programs and for identifying the most reliable traits for selection for improved grain yield under drought and low N conditions.

2. MATERIALS AND METHODS

2.1 Plant Materials

Seeds of 19 maize (Zea mays L.) genotypes, namely nine single crosses (SC-10, SC-131, SC-168 and SC-176 from Agricultural Research Center; ARC, SC-30K8 and SC-30N11, from Pioneer-Corteva Agriscience, SC-2031 and SC-2055 from Hi-Tec Company and SC-101 from Fine Seeds Company), five three-way crosses (TWC-310, TWC-321, TWC-352, TWC-360 from ARC and TWC-1100 from Hi-Tec Company) and five open-pollinated populations from ARC (American Early Dent; AED, Giza 2 Synthetic and Nubaria-355 Synthetic of Egyptian origin, and Original Midland and Reid Type Composite of USA origin). The grain color is white for 11 genotypes (SC-10, 30K8, SC-101, SC-131, SC-2031, TWC-310, TWC-321, TWC-1100, AED, Giza-2 and Nubaria) and yellow for the rest of genotypes.

2.2 Experimental Procedure

This study was carried out in the two successive growing seasons 2016 and 2017 at the Agricultural Experiment and Research Station of the Faculty of Agriculture, Cairo University, Giza, Egypt (30° 02’N latitude and 31° 13’E longitude with an altitude of 22.50 meters above sea level). Sowing date was April 24th in the 1st season (2016) and April 30th in the 2nd season (2017).
Sowing was done in rows; each row was 4 m long and 0.7 m width. Seeds were over sown in hills 25 cm apart, thereafter (after 21 days from planting and before the 1st irrigation) were thinned to one plant/hill to achieve a plant density of about 57,120 plants/ha. Each experimental plot included two rows (plot size = 4×1.4=5.6 m²).

Evaluation in each season was carried out under 6 environments (from E1 to E6), i.e., three nitrogen levels, i.e., high-N (HN), medium-N (MN) and low-N (LN) by adding 285.6, 166.6 and 47.6 kg N/ha, respectively in two equal doses in the form of Urea 46% before 1st and 2nd irrigations and two irrigation regimes, i.e. well-watered (WW) and water stressed (WS) conditions as follows: E1: High nitrogen-well watered (HN-WW), E2: High nitrogen-water stress (HN-WS), E3: Medium nitrogen-well watered (MN-WW), E4: Medium nitrogen-water stress (MN-WS), E5: Low nitrogen-well watered (LN-WW) and E6: Low nitrogen-water stress (LN-WS).

2.3 Experimental Design

A split-split-plot design in randomized complete blocks arrangement with three replications was used. Main plots were allotted to two irrigation regimes, i.e. well-watered (WW) and water stressed treatments at flowering (WS). Each main plot was surrounded with an alley (4 m width), to avoid water leaching between plots. Sub-plots were assigned to three nitrogen fertilizer rates, i.e. 47.6, 166.6 and 285.6 kg N/ha, respectively. Sub-sub-plots were devoted to nineteen maize genotypes.

2.4 Water Regimes

The following two different water regimes were used: 1. Well-watered regime (WW): The recommended well irrigation method by ARC for the whole season was applied, the irrigations were given every 12-15 days. 2. Water stressed regime (WS) at flowering: The irrigation regime was just like WW, but the 4th and 5th irrigations were withheld, resulting in 24 days’ water stress just before and during the flowering stage.

2.5 Fertilization Regimes

Nitrogen fertilization for each rate was added in two equal doses of Urea 46% before the first and second irrigation. Triple Superphosphate Fertilizer (46% P₂O₅) at the rate of 30 kg P₂O₅/fed (70 kg P₂O₅/ha), was added as soil application before sowing during the preparation of the soil for planting.

Weed control was performed chemically with Stomp herbicide just after sowing the seed and before the sowing irrigation and manually by hoeing twice, the first before the first irrigation (after 21 days from sowing) and the second before the second irrigation (after 33 days from sowing). Pest control was performed when required by spraying plants with Lannate (Methomyl) 90% (manufactured by DuPont, USA) against corn borers. All other agricultural practices were followed according to the recommendations of ARC, Egypt.

2.6 Soil Analysis

Physical and chemical soil analyses of the field experiments were performed at laboratories of Soil and Water Research Institute of ARC, Egypt. The soil type is clay loam (39.48% silt, 35.73% clay, 18.07% fine sand and 6.72% coarse sand as an average of the two seasons). The soil pH (paste extract) was 7.93; the EC was 2.23 dSm⁻¹. Available soil nitrogen in 30 cm depth was analyzed immediately prior to sowing at the laboratories of Water and Environment Unit, ARC, Egypt and found to be 148.0 and 7.2 kg N/ha in 2016 and 2017 seasons, respectively. Available soil nitrogen after adding nitrogen fertilizer was therefore 433.6, 314.6 and 195.6 kg N/ha in the 1st season and 358.2, 239.2 and 120.2 kg N/ha, in the 2nd season for the 3 N treatments, i.e. HN, MN, and LN, respectively. The available nitrogen to each plant (including soil and added N) was calculated for each environment and found to be 7.59, 5.51 and 3.42 g N/plant in the first season and 6.27, 4.19 and 2.10 g N/plant in the second season, with an average across the two seasons of 6.93, 4.85 and 2.76 g N/plant for the three N treatments, respectively.

2.7 Meteorological Data

The required weather data for the experimental site through the two growing seasons were obtained from Central Lab for Agricultural Climate, Agricultural Research Center at Giza, Governorate, Egypt. Mean temperature in May, June, July and August was 28.9, 33.5, 32.6 and 32.5°C in 2016 season and 29.3, 23.3, 33.5 and 32.5°C in 2017 season. Relative humidity was 38.7, 31.7, 46.3 and 44.3% in 2016 season and 34.0, 23.3, 42.3 and 46.3% in 2017 season. Sunshine duration was 13.4, 13.9, 13.8 and 13.0 hr in 2016 season and 13.4, 13.9, 13.8 and 13.1 hr in 2017 season.
2.8 Morphological Data Recorded

1) Days to 50% tasselling (DTA), 2) Days to 50% silking (DTS), 3) Anthesis-silking interval (ASI), 4) Plant height (PH), 5) Ear height (EH), 6) Chlorophyll concentration index (CCI) by Chlorophyll Concentration Meter, Model CCM-200, USA (available on line at: http://www.apogeeinstruments.co.uk/apogee-instruments-chlorophyll-content-meter-technical-information/), 7) Number of ears plant\(^{-1}\) (EPP), 8) Number of rows ear\(^{-1}\) (RPE), 9) Number of kernels row\(^{-1}\) (KPR), 10) Number of kernels plant\(^{-1}\) (KPP), 11) 100-kernel weight (HKW) (g), 12) Grain yield plant\(^{-1}\) (GYPP) (g) (adjusted at 15.5% grain moisture), 13) Economic nitrogen use efficiency (NUE\(_e\)) (g/g) as follows: NUE\(_e\) = GDM/Ns, where GDM = grain dry matter, Ns = available soil-N/plant, 14) Grain nitrogen content (GN) (in g), 15) Grain nitrogen utilization efficiency (NUTE\(_g\)) (g/g) as follows: NUTE\(_g\) = (GDM/GN), 16) Grain protein content (GPC) in %, 17) Grain starch content (GSC) in %, 18) Grain oil content (GOC) in % and 19) Grain fiber content (GFC) in %. The grain quality traits (GPC, GSC, GOC and GFC) were measured in both seasons, on samples taken from the grain bulk of each maize genotype by using INSTALAB 600 Near Infrared (NIR) Product Analyzer manufactured by DICKEY-john Corporation, Auburn, Illinois, USA.

2.9 Biometrical Analysis

Analysis of variance of the split-split-plot design each year was computed on the basis of individual plot observation using the MIXED procedure of MSTAT \(®\). A combined analysis of variance of the split-split-plot design across the two years was also performed if the homogeneity test was non-significant. Moreover, each of the six environments was analyzed separately as a randomized complete block design (RCBD) for the purpose of determining genetic parameters, i.e. under WW-HN (non-stressed environment), WW-MN, WW-LN (low N stress environment), WS-HN (drought stress environment), WS-MN, and WS-LN (drought combined with low N environment). LSD values were calculated to test the significance of differences between means according to Steel et al. [37].

2.10 Morphological Evaluations

The best use of the information contained in the data for morphological characterization is an important issue in plant breeding. To display the genetic variability among maize genotypes, a Genotype × Trait biplot (GT biplot) of standardized data was applied for each of the three environments drought stressed environment (WS-HN), low N stressed environment (WW-LN) and drought combined with low N stressed environment (WS-LN). To generate a GT biplot [38], the genotype by trait two-way table of data was first trait-standardized. The standardization is necessary to remove the units, because different traits use different units. The trait-standardized table (data standardized) was then decomposed into principal components (PC). The first two PC’s (PC1 and PC2) were used to generate a GT biplot. PC1 and PC2 were scaled so that values are symmetrically distributed between the genotype scores and trait scores. A genotype by trait biplot is constructed by plotting the PC1 scores against the PC2 scores for each genotype and each trait. The biplot technique provides a powerful tool for data analysis of genotype × trait data in individual environments and can be used to visualize the genetic correlations among traits and evaluation of the genotype on the basis of multiple traits [39]. The GT biplot software XLSTAT [40] was used for all calculations.

3. RESULTS

3.1 Phenotypic Identification and Variation

Analysis of variance (ANOVA) of split-split plot design (data not presented) indicated that mean squares due to genotype (G), were significant (\(p \leq 0.01\)) for all studied traits. ANOVA of randomized complete block design for each (separate) environment (drought, low N or drought combined with low N stress) indicated that mean squares due to genotypes across two years were significant (\(p \leq 0.01\)), suggesting significant differences among 19 maize hybrids and populations for all studied 19 traits (Table 1). Coefficient of variation (CV) was generally very low (≤10%) for all studied traits, except for anthesis-silking interval (ASI), which was 22.6, 20.6 and 31.2% under drought, low N and drought combined with low N stressed environments, respectively, indicating good accuracy of the experiment.

The variability among maize genotypes expressed by phenotypic coefficient of variation (PCV) was the highest for chlorophyll concentration index (CCI) (82.3%), ASI (113.8%), and NUE (103.5%), under drought, low
N and drought combined with low N stressed environments, respectively followed by ASI, GFC, NUE and GYPP under drought, NUE and GYPP under low N and GYPP and GOC under drought combined with low N stressed environment (Table 1). On the contrary, the lowest PCV was recorded by grain starch content (6.0, 5.2 and 8.1%) under drought, low N and drought combined with low N stressed environments, respectively.

Mean grain yield/plant ranged from 98.0 to 217.6 g with an average of 150.6 g under droughted environment, from 66.4 to 229.7 g under low N stressed environment and from 40.6 to 157.9 g under drought combined with low N stressed environment (Table 1). The single cross hybrid SC-101 (G3) had the highest grain yield, the highest 100-kernel weight (40.47, 41.97 and 39.97 g) under the three environments, respectively and the highest NUEe (83.22 and 57.21 g/g) under the two environments low N and drought combined with low N and the highest GSC under low N and the highest NUTEg under drought combined with low N environment.

Mean number of days to 50% anthesis ranged between 58.2 and 68.8 with an average of 63.4 for droughted environment, from 57.5 to 69.0 with an average of 62.7 day for low N environment and from 58.8 to 70.7 with an average of 64.3 day for drought combined with low N environment. Days to 50% silking ranged between 61.87 and 73.63 with an average of 67.87 for drought environment, from 61.63 to 73.67 with an average of 66.9 day for low N environment and from 64.13 to 76.03 with an average of 69.45 day for drought combined with low N environment. Anthesis silking interval ranged from 2.17 to 5.33 day with an average of 3.98 day for drought environment, from 2.50 to 9.67 with an average of 4.2 day for low N environment and from 3.67 to 7.0 with an average of 5.15 day for drought combined with low N environment. Genotype G6 (SC-30N11) had the latest anthesis and latest silking, the highest ear position, the highest CCI, under the three environments.

The tallest plant was recorded by G1 (SC-10) under drought and G6 (SC-30N11) under the two environments low N and drought combined with low N, but the shortest plant was recorded by G13 (TWC-352) under drought and G18 (Midland) under the two environments low N and drought combined with low N. The highest grain protein content was recorded by G6 (SC-30N11) under the two environments low N and drought combined with low N and G18 (Midland) under drought combined with low N environment. The highest grain oil content, was recorded by genotype G9 (SC-2055) under drought environment, G13 (TWC-352) under low N and G6 (SC-30N11) under drought combined with low N environment.

### 3.2 Principal Component Analysis

To display the genetic variability among maize genotypes under the three environments drought, low N and drought combined with low N, a principal component analysis of standardized data was applied to display maize trait relationships, and its application in genotype characterization and comparison (Table 2). Based on different traits use different units, the data standardization is necessary to remove the units. Principal components, PC1 (F1) and PC2 (F2) were scaled so that values are symmetrically distributed between the genotype scores and trait scores. A genotype by trait biplot is constructed by plotting the PC1 scores for each genotype (19) and each trait (19). The genotype by trait biplot effectively reveals the interrelationships among maize traits (Figs. 1-3). GT-biplot also provides a tool for visual comparison among genotypes based on multiple traits. The results of the genotype by trait biplot, explained 49.48, 56.99 and 54.69% of the total variation for the three environments drought, low N and drought combined with low N, respectively, and are a good approximation of the total variation of the standardization data.

Biplot in the principal component represents variables that are super imposed on a plot as vectors where relative length of vectors represents the relative proportion of variability in each variable represented on biplot. Based on PC1 and PC2, 100-kernel weight, ears/plant and ear height followed by grain yield/plant, NUE, plant height, rows/ear, kernels/plant, grain protein content and grain oil content under drought stress, GYPP, NUE, 100-KW, RPE, ASI, EH, PH, GPC, DTA, DTS and NUTE under low N environment and GYPP, NUE, DTA, DTS, NUTE, EH, 100-KW, RPE, CCI, GPC and GSC under drought combined with low N environment had relatively long vectors, suggesting that there was relatively large variation among genotypes. In other words, they show large variation among the 19 genotypes studied, suggesting that they are the most discriminator of the morphological data under the respective environments.
Table 1. Summary statistics for 19 phenotypic traits of 19 maize genotypes evaluated in the field under drought, low N and drought combined with low N across two seasons

| Trait        | Minimum       | Maximum       | Mean   | LSD\_0.05 (Genotype) | CV\%  | R\(^2\) | MS(Genotype) | PCV\%  |
|--------------|---------------|---------------|--------|----------------------|-------|---------|--------------|--------|
| Under drought|               |               |        |                      |       |         |              |        |
| DTA          | 58.2(18)      | 68.8(6)       | 63.4   | 1.9                  | 2.9   | 0.89    | **           | 13.2   |
| DTS          | 61.87(8)      | 73.63(6)      | 67.87  | 1.91                 | 3.0   | 0.91    | **           | 13.2   |
| ASI          | 2.17(5)       | 5.33 (15)     | 3.98   | 1.17                 | 22.6  | 0.78    | **           | 78.3   |
| PH (cm)      | 216.7(13)     | 270.8(1)      | 243.1  | 12.94                | 6.7   | 0.79    | **           | 19.4   |
| EH (cm)      | 100.8(18)     | 140.3(6)      | 116.6  | 6.09                 | 9.6   | 0.73    | **           | 24.2   |
| CCI (%)      | 12.62(8)      | 34.96(6)      | 20.45  | 1.54                 | 9.3   | 0.96    | **           | 82.3   |
| EPP          | 0.783(19)     | 1.15(5)       | 1.013  | 0.07                 | 5.3   | 0.93    | **           | 39.5   |
| RPE          | 11.71(3)      | 16.01 (15)    | 13.86  | 0.28                 | 4.4   | 0.90    | **           | 21.9   |
| KPR          | 32.7(6)       | 45.7(2)       | 39.7   | 1.5                  | 3.9   | 0.95    | **           | 27.0   |
| KPP          | 458(14)       | 684 (9)       | 559    | 17.9                 | 3.2   | 0.98    | **           | 38.6   |
| 100-KW (g)   | 25.21(19)     | 40.47(3)      | 32.43  | 2.55                 | 7.9   | 0.83    | **           | 31.5   |
| GYPP (g)     | 98.0(19)      | 217.6(3)      | 150.6  | 7.33                 | 5.4   | 0.99    | **           | 69.8   |
| GN (g)       | 0.982(1)      | 2.933(16)     | 1.802  | 0.141                | 9.4   | 0.97    | **           | 20.6   |
| NUEe (g/g)   | 14.14(15)     | 31.4(6)       | 24.45  | 1.14                 | 6.2   | 0.99    | **           | 72.2   |
| NUTEg (g/g)  | 55.06(7)      | 85.38(2)      | 74.4   | 7.52                 | 9.9   | 0.82    | **           | 18.9   |
| GPC (%)      | 7.42(4)       | 9.87(6)       | 8.39   | 0.86                 | 4.2   | 0.92    | **           | 20.4   |
| GOC (%)      | 1.89(6)       | 4.22(9)       | 2.75   | 0.5                  | 6.7   | 0.84    | **           | 63.9   |
| GSC (%)      | 62.57(9)      | 68.22(19)     | 66.35  | 1.15                 | 2.5   | 0.87    | **           | 6.0    |
| GFC (%)      | 0.84(6)       | 2.58(15)      | 1.68   | 0.34                 | 9.9   | 0.80    | **           | 73.6   |
| Under low N  |               |               |        |                      |       |         |              |        |
| DTA          | 57.5(19)      | 69.0(6)       | 62.7   | 2.2                  | 3.2   | 0.91    | **           | 11.8   |
| DTS          | 61.63(16)     | 73.67(6)      | 66.9   | 2.2                  | 3.3   | 0.93    | **           | 10.4   |
| ASI          | 2.50(1)       | 9.67 (15)     | 4.2    | 1.62                 | 20.6  | 0.76    | **           | 113.8  |
| PH (cm)      | 211.5(18)     | 286(6)        | 242.5  | 18.37                | 6.6   | 0.81    | **           | 19.2   |
| EH (cm)      | 94.7(18)      | 140.8(6)      | 112.2  | 8.37                 | 8.9   | 0.78    | **           | 28.3   |
| CCI (%)      | 10.66(12)     | 14.53(6)      | 12.27  | 1.19                 | 9.5   | 0.98    | **           | 33.1   |
| EPP          | 0.762(15)     | 1.121(2)      | 0.959  | 0.05                 | 5.7   | 0.91    | **           | 26.8   |
| RPE          | 11.42(5)      | 15.78 (18)    | 13.86  | 0.61                 | 4.2   | 0.93    | **           | 26.3   |
| KPR          | 33.58(19)     | 44.58(12)     | 39.54  | 1.8                  | 3.7   | 0.95    | **           | 33.1   |
| KPP          | 338.6(6)      | 664(2)        | 520.7  | 14.0                 | 3.9   | 0.96    | **           | 41.9   |
| Trait          | Minimum   | Maximum   | Mean    | LSD<sub>0.05 (Genotype)</sub> | CV% | R<sup>2</sup> | MS (Genotype) | PCV% |
|---------------|-----------|-----------|---------|-------------------------------|-----|-------------|--------------|------|
| **Under drought** |           |           |         |                               |     |             |              |      |
| 100-KW(g)     | 25.68(13) | 41.97(3)  | 32.04   | 2.51                          | 8.2 | 0.87       | **           | 32.6 |
| GYPP (g)      | 66.4(19)  | 229.7(3)  | 143     | 5.98                          | 5.6 | 0.97       | **           | 76.3 |
| GN (g)        | 0.663(18) | 2.138(3)  | 1.55    | 0.09                          | 8.6 | 0.91       | **           | 30.4 |
| NUTEe (g/g)   | 24.06(19) | 83.22(3)  | 21.74   | 2.64                          | 5.7 | 0.98       | **           | 86.2 |
| NUTEg (g/g)   | 76.61(6)  | 109.49(2) | 94.56   | 6.94                          | 7.8 | 0.85       | **           | 27.0 |
| GPC (%)       | 5.77(2)   | 9.45(6)   | 6.84    | 0.59                          | 4.6 | 0.94       | **           | 30.4 |
| GOC (%)       | 1.82(8)   | 4.21(13)  | 2.95    | 0.61                          | 6.9 | 0.87       | **           | 46.7 |
| GSC (%)       | 64.57(13) | 70.32(3)  | 67.78   | 1.15                          | 2.9 | 0.88       | **           | 5.2  |
| GFC (%)       | 1.00(3)   | 2.14(10)  | 1.64    | 0.34                          | 9.9 | 0.83       | **           | 49   |
| **Under drought combined with low N** |           |           |         |                               |     |             |              |      |
| DTA           | 58.8(19)  | 70.7(6)   | 64.3    | 2.5                           | 2.8 | 0.92       | **           | 13.1 |
| DTS           | 64.13(3)  | 76.03(6)  | 69.45   | 2.5                           | 2.9 | 0.93       | **           | 10.9 |
| ASI           | 3.67(5)   | 7.0(15)   | 5.15    | 1.53                          | 31.2| 0.75       | **           | 55.4 |
| PH (cm)       | 198.6(13) | 264.2(6)  | 228.8   | 20.7                          | 6.9 | 0.73       | **           | 21.0 |
| EH (cm)       | 89.2(18)  | 137.2(6)  | 109     | 10.7                          | 8.7 | 0.71       | **           | 24.3 |
| CCI (%)       | 10.6(1)   | 22.38(6)  | 14.0    | 1.51                          | 8.9 | 0.94       | **           | 58.9 |
| EPP           | 0.718(13) | 0.994(6)  | 0.879   | 0.05                          | 6.2 | 0.91       | **           | 24.3 |
| RPE           | 10.88(5)  | 14.92(9)  | 13.25   | 0.66                          | 4.5 | 0.93       | **           | 24.8 |
| KPR           | 27.56(6)  | 42.17(1)  | 35.99   | 1.87                          | 4.0 | 0.96       | **           | 24.6 |
| KPP           | 315.5(14) | 563(2)    | 441     | 17.2                          | 3.3 | 0.99       | **           | 34.0 |
| 100-KW(g)     | 24.28(19) | 39.97(3)  | 30.06   | 3.75                          | 7.6 | 0.81       | **           | 30.4 |
| GYPP (g)      | 40.6 (19) | 157.9(3)  | 97.1    | 8.72                          | 6.5 | 0.98       | **           | 83.5 |
| GN (g)        | 0.73(14)  | 1.59(7)   | 1.183   | 0.1                           | 10.0| 0.96       | **           | 33.8 |
| NUEe(g/g)     | 14.71(19) | 57.21(3)  | 35.0    | 4.0                           | 5.9 | 0.97       | **           | 103.5 |
| NUTEg(g/g)    | 75.02(18) | 142.46(3) | 92.95   | 16.6                          | 8.8 | 0.84       | **           | 48.6 |
| GPC (%)       | 4.94(3)   | 8.54(18)  | 7.09    | 0.66                          | 5.6 | 0.95       | **           | 33.8 |
| GOC (%)       | 1.71(14)  | 4.58(6)   | 3.26    | 0.82                          | 10.5| 0.81       | **           | 66.7 |
| GSC (%)       | 62.54(6)  | 70.45(1)  | 67.34   | 2.27                          | 3.4 | 0.8        | **           | 8.1  |
| GFC (%)       | 1.15(3)   | 2.42(16)  | 1.79    | 0.45                          | 10.3| 0.91       | **           | 58.3 |

Values are followed by genotype (G) No. in parenthesis. MS = Mean squares from ANOVA. CV = coefficient of variation, ** indicate significance at 0.01 probability level.
Table 2. Principal component analysis (PCA) for morphological data, under drought, low N and drought combined with low N across two years

| Trait | Under drought | Under low N | Under drought combined with low N |
|-------|---------------|-------------|----------------------------------|
|       | F1    | F2  | F3  | F1    | F2  | F3  | F1    | F2  | F3  |
| GYPP  | 0.74  | -0.01| -0.09| 0.89  | -0.24| -0.14| 0.9   | -0.03| 0.18|
| NUE   | 0.74  | -0.01| -0.09| 0.89  | -0.23| -0.14| 0.9   | -0.03| 0.18|
| GN    | 0.34  | 0.18 | -0.52| 0.78  | -0.12| -0.15| 0.43  | -0.09| 0.51|
| 100GW | 0.76  | -0.02| 0.27 | 0.83  | 0.03 | -0.12| 0.75  | 0.23 | 0.00|
| DTA   | 0.63  | -0.23| -0.22| 0.51  | 0.70 | 0.22 | 0.16  | 0.83 | -0.39|
| DTS   | 0.63  | -0.23| -0.22| 0.51  | 0.70 | 0.22 | 0.16  | 0.83 | -0.39|
| ASI   | -0.48 | -0.25| -0.27| -0.77| 0.10 | -0.2 | -0.63 | -0.15| 0.39|
| PH    | 0.72  | 0.04 | -0.05| 0.53  | 0.74 | 0.02 | 0.53  | 0.56 | 0.10|
| EH    | 0.76  | -0.24| -0.21| 0.44  | 0.77 | 0.15 | 0.45  | 0.74 | -0.01|
| CCI   | 0.24  | -0.51| -0.25| 0.26  | 0.00 | -0.38| 0.34  | 0.61 | 0.18|
| NUTE  | 0.25  | 0.69 | 0.48 | 0.45  | -0.64| 0.36 | 0.8   | -0.34| -0.15|
| EPP   | 0.76  | 0.05 | -0.1 | 0.69  | -0.01| 0.02 | 0.51  | 0.32 | 0.49|
| RPE   | -0.73 | 0.21 | -0.52| -0.79 | -0.17| 0.09 | 0.62  | -0.44| 0.23|
| KPR   | 0.20  | 0.61 | 0.54 | 0.76  | -0.25| 0.37 | 0.64  | -0.28| 0.28|
| KPP   | 0.38  | 0.71 | -0.4 | 0.29  | -0.61| 0.52 | 0.51  | -0.25| 0.64|
| GPC   | -0.25 | -0.75| -0.49| -0.25| 0.73 | -0.46| -0.76 | 0.31 | 0.14|
| GOC   | -0.14 | 0.75 | -0.52| -0.11| 0.14 | 0.9 | -0.23 | 0.48 | 0.69|
| GSC   | -0.24 | -0.59| 0.68 | 0.26  | -0.52| -0.64| 0.33  | -0.63| -0.34|
| GFC   | -0.58 | 0.44 | -0.18| -0.47| 0.06 | 0.58 | -0.5  | 0.28 | 0.56|

|   | Eigenvalue | Variability (%) | Cumulative % |
|---|------------|-----------------|--------------|
|   | 5.83       | 30.68           | 30.68        |
|   | 3.57       | 18.8            | 49.48        |
|   | 2.6        | 13.7            | 63.17        |
|   | 6.87       | 36.17           | 56.99        |
|   | 3.95       | 20.81           | 71.2         |
|   | 2.7        | 14.21           | 33.39        |
|   | 5.83       | 33.39           | 54.69        |
|   | 3.57       | 21.31           | 72.39        |

Eigenvalue | Variability (%) | Cumulative % |
On the contrary, GN, ASI, CCI and GFC under drought, CCI, GOC, GSC, GFC under low N and GN, EPP, KPP, GOC, GFC and ASI under drought combined with low N stress were the least discriminator, based on both PC1 and PC2 (Table 2 and Figs. 1 to 3). Grain yield/plant, followed by NUE, 100-KW, EPP, PH, and EH under drought, GYPF followed by NUE, 100-KW, GN, ASI, RPE, KPR under low N, and GYPF, NUE, 100-KW, ASI, EPP, GPC, KPR and RPE under drought combined with low N based on PC1 only, NUTE, KPR, KPP, GPC, GOC and GSC under drought, DTA, DTS, PH, EH, NUTE, KPP and GOC under low N and DTA, DTS, EH, CCI, and GSC under drought combined with low N stress based on PC2 only are the most discriminator of the studied morphological traits.

The cosine of the angle between the vectors of two traits measures the similarity or the correlation between them relative to their variation among genotypes. Thus, an angle of zero indicates a correlation of +1, an angle < 90° suggests a positive correlation, an angle of 90° indicates no (0) correlation, implying independence, an angle > 90° indicates negative correlation, and an angle of 180° represents a correlation of -1.

Each of the Figs. (1, 2 and 3) showed four groups of traits versus four groups of genotypes; each group is characterized with high values in the respective traits. Under drought environment (Fig. 1), GYPF, 100-KW, NUE, EH, DTA, DTS, and CCI for the genotypes G3, G5, G1 and G6 in the first group, EPP, PH, GN, KPP, NUTE and KPR for the genotypes G2, G4, G11, G16, G12, and G9 in the second group, GOC, GPC and RPE for the genotypes G13, G15 and G17 in the third group, ASI, GSC and GPC for the genotypes G8, G10, G7, G14, G18 and G19 in the 4th group.

Under low N environment (Fig. 2), GYPF, NUE, KPR, GN, EPP, CCI, NUTE, KPP and GSC for the genotypes G3, G2, G4, G17, G14 and G9 in the first group, 100-KW, DTA, DTS, PH, and EH for the genotypes G1, G5, G11, G10, G12, and G6 in the second group, GPC, GOC, GFC and ASI for the genotypes G13 and G15 in the third group, RPE for the genotypes G16, G17, G8, G18 and G19 in the 4th group.

Under drought combined with low N environment (Fig. 3), GYPF, NUE, GN, KPP, KPR, NUTE and GSC for the genotypes G3, G2, G1, G4, G17, G8 and G9 in the first group, DTA, DTS, EH, PH, EPP and 100-KW for the genotypes G10, G12, and G5 in the second group, GOC, GFC, CCI, and GPC for the genotypes G6, G7 and G11 in the third group, ASI, and RPE for the genotypes G13, G14, G15, G16, G18 and G19 in the 4th group.

The traits pair GYPF and NUE had an angle of zero under the three studied environments (drought, low N and drought combined with low N), indicating a perfect correlation of +1. Traits of each group had acute (< 90°) angles between them, indicating that their variation were similar, so each trait inside a specific group can be recorded instead of the other trait in the same group; namely under drought environment, GYPF, 100-KW, NUE, EH, DTA, DTS, and CCI in the 1st group, EPP, PH, GN, KPP, NUTE and KPR in the second group, GOC, GFC and RPE in the third group, ASI, GSC and GPC in the 4th group, under low N environment, GYPF, NUE, KPR, GN, EPP, CCI, NUTE, KPP and GSC in the first group, 100-KW, DTA, DTS, PH, and EH in the second group, GPC, GOC, GFC and ASI in the third group, RPE in the 4th group and under drought combined with low N environment (Fig. 3), GYPF, NUE, GN, KPP, KPR, NUTE and GSC in the first group, DTA, DTS, EH, PH, EPP and 100-KW in the second group, GOC, GFC, CCI, and GPC in the third group, ASI, and RPE in the 4th group. The traits of each group are inter-correlated.

Genotype by trait biplot figures indicated that grain yield/plant (or NUE) are positively correlated with 100-KW, EH, DTA, DTS, EPP, PH, GN and CCI and negatively correlated with ASI, RPE and GFC under drought environment, positively correlated with KPR, GN, EPP, 100-KW, CCI, NUTE, KPP and GSC and negatively correlated with ASI, RPE, GOC, GPC and GFC under low N environment and positively correlated with GN, KPR, KPP, NUTE, EPP, 100-KW, and GSC and negatively correlated with ASI, RPE, GOC, GPC, CCI and GFC under drought combined with low N environment. These traits could be considered selection criteria for grain yield (stress tolerance) or for nitrogen use efficiency under respective stressed environments if the heritability and genetic advance from selection of these traits are high; the common selection criteria are 100-KW, EPP, GN, ASI, RPE an GFC in the three studied environments and KPR, NUTE, GSC, GOC and GPC in the two environments low N and drought combined with low N.
Fig. 1. Genotype by trait biplot illustrating the relationship between PC1 and PC2 for 19 genotypes and 19 traits of maize under water stress conditions.

Fig. 2. Genotype by trait biplot illustrating the relationship between PC1 and PC2 for 19 genotypes and 19 traits of maize under low N stress conditions.
Fig. 3. Genotype by trait biplot illustrating the relationship between PC1 and PC2 for 19 genotypes and 19 traits of maize under drought combined with low N stress conditions.

Trait pairs GYPP vs DTA, DTS, PH and EH under low N environment and GYPP (or NUE) vs DTA and DTS under drought combined with low N had a near-right angle, indicating that variation of one trait was more or less independent of the other trait (near zero correlation). On the contrary, GYPP (or NUE) vs RPE, GFC, GPC, GOC, ASI (under drought environment) vs GPC, GOC, under low N environment and vs GOC, CCI, GFC, RPE and GOC under drought combined with low N environment, had obtuse (>90°) angles, indicating that their variation was in opposite directions (negative correlation). Trait pairs GYPP vs ASI under low N environment had an angle of 180° representing a perfect negative correlation of -1.

4. DISCUSSION

The present investigation studied 19 maize genotypes by 19 phenotypic traits. The presence of genetic variability among the studied genotypes for grain yield and other studied traits under water stress and low N conditions suggested that significant progress could be achieved by selection for improved grain yield and other traits under water stressed and low N conditions. Although morphological assessment of genetic diversity presents many limitations as low polymorphism and environmental influence on phenotypic performance [41], phenotypic traits were useful as a preliminary assessment of maize genetic variability and provided practical and critical information required to characterize germplasm collections [42,43].

Morphological traits measured (19 traits) depicted significant (p≤0.01) differences among the maize genotypes under study. Variation for most studied traits was observed. Morphological traits are very important for classifying maize germplasm, and also are helpful and essential for maize breeders seeking to improve existing germplasm by introducing novel genetic variation for certain traits into the breeding populations [8,13,44-46]. They reported the existence of
substantial variability in different gene pools of maize germplasm.

Coefficient of variation (CV) was generally very low (<10%) for most of studied traits, indicating the accuracy in implementing the experiment. The exception was ASI, where CV was 22.6, 20.6 and 31.2% under drought, low N and drought combined with low N stressed environments, respectively. The large CV in this study for ASI is in agreement with results reported by Asare et al. [44] and Twumasi et al. [45].

The high phenotypic coefficient of variability (PCV) was observed, particularly for the traits CCI, ASI, GFC, NUE and GYPP under drought, ASI, NUE, and GYPP under low N and NUE, GYPP and GOC under drought combined with low N stressed environment. Sultana [47] also recorded significant (ps0.01) differences for these traits. Sharma et al. [48] reported significant differences among 20 maize genotypes for days to 50% flowering, cob length, plant height, 100-grain weight and grain yield per plant. Saeed et al. [49] found significant differences among genotypes provided by CIMMYT and local checks for all studied traits, except ear height. Such high variability suggested that the germplasm was adapted to a wide range of environmental conditions, and could provide valuable alleles for maize improvement [13,50].

Variability in ASI, NUE and GYPP was about six to thirteen fold larger than variability in GSC, DTA and DTS, in the studied genotypes of maize. Such a large phenotypic variability, reflects the differential fitness to the environment, flexibility and survival in changing environmental conditions. The data obtained would help parental selection and broadening the genetic base of maize breeding populations [45,46]. The presence of genetic variability among the genotypes for grain yield and other agronomic traits under drought, low N or drought combined with low N stress conditions indicated that significant progress could be achieved in selecting for improved grain yield and other traits under these stressed conditions [30].

The highest GYPP, 100-KW under the three environments, and the highest NUEe under low N and drought combined with low N, the highest GSC under low N and the highest NUTEg under drought combined with low N environment were shown by the genotype G3, while the highest grain protein content, grain oil content, ears/plant and the shortest ASI were recorded by G6, G9 and G5 under drought, G6, G13, G2 and G1 under low N and G18, G6, G6 and G5 under drought combined with low N, respectively. Genotype G2 was also the best in NUTEg and kernels/row under drought, NUTEg and kernels/plant under low N and kernels/plant under drought combined with low N. These genotypes might carry favorable genes that could be exploited to improve Egyptian maize for the respective traits under the respective stressed environments.

Various studies have employed multivariate analysis such as PCA to evaluate the magnitude of genetic diversity among the crop germplasm [51] and to reduce a large number of observed traits into a smaller set of traits that have the maximum contribution in separating the genotypes. The PCA was performed in the present study to classify the maize genotypes on the basis of the most discriminating traits. Our results revealed two main components accounting for 49.48, 56.99 and 54.69% of total variability under drought, low N and drought combined with low N stresses, respectively. In general, the contribution of PC1 for the 19 traits were 1.63, 1.74 and 1.57 fold more than that of PC2, under drought, low N and drought combined with low N stresses, respectively. Bin Mustafa et al. [52] reported that 59.3 and 55% of the total variation were contributed by the first two PCs when evaluating maize genotypes at 100 and 40% moisture levels. PCA showed that the three factors had eigenvalue > 1, moreover 56.13 and 57.2; 56.22 and 58.94% of the total variability were explained by the first two PCs under the same conditions. Others recorded two PCs that contributed to 94.01% and 91.15% of total variation in root traits of 103 maize inbred lines evaluated under control and water-stressed conditions [53]. Suryanarayana et al. [54] applied PCA and cluster analysis to data for 30 maize accessions in India. In their study the first five PCs had Eigen values greater than 1, which collectively explaining 85.31% of the variance. They identified days to 50% anthesis, ear length and number of kernels per row as the factors that made the greatest contributions to total variance. Pahadi et al. [55] found eigenvalues greater than 1 for their first two PCs, which explained 73.7% of the variance. They noted that days to 50% anthesis was the most influential trait. Ali et al. [56] found eigenvalues greater than 1 for their first three PCs, which accounted for 89.60% of the variance in maize hybrids grown in Pakistan.
Kumari et al. [8] found that the first two principal components explained more than 50% of the phenotypic variation among collected landraces and traditional cultivars of maize. Saeed et al. [49] found four PCs had eigenvalues greater than 1 and explained 73.38% of the total variance in maize genotypes provided by CIMMYT and in local checks in Pakistan. 

This result is in agreement with the findings of other investigators [29,30] who identified EPP, plant and ear aspects, days to silking, and plant and ear heights as the most reliable for improved grain yield under drought and low N. Badu-Apraku et al. [29] reported that the most reliable traits for selection for improved grain yield under drought in the early maturing germplasm were ear aspect, ears per plant, anthesis-silking interval, and plant aspect. Badu-Apraku et al [59] identified plant aspect and plant and ear heights as the most reliable traits for simultaneous selection for yield under low N and drought in the extra-early inbreds. Oyekunle et al. [60] identified plant and ear aspects, ear height, stay green characteristic, number of seeds per ear, and number of seeds per row were identified by GGE biplot analyses as the most reliable traits for selecting for grain yield under drought.

Similarly, the existence of negative correlations between grain yield (or NUE) and each of ASI, RPE and GFC under drought environment, ASI, RPE, GOc, GPC and GFC under low N environment and ASI, RPE, GOc, GPC, CCI and GFC under drought combined with low N conditions indicate that these traits might have direct or indirect effects on grain yield under these conditions. These results justified the inclusion of most of these traits in the base index for selection of genotypes for tolerance to drought and/or low N stresses. Several studies have identified yield components as strong secondary traits for yield improvement under drought or/and low N stresses due to the strong genetic correlations with yield under such stress conditions. On the contrary, results of the GT biplot in the present study indicated that short ASI was the most reliable secondary trait for improving grain yield under drought and/or low N stressed conditions. These results are in accordance with those reported by other investigators [29,33,61-64].

Our results are in agreement with the findings of Aci et al. [50], who reported that maize landraces characterized by short ASI were the most productive. An ASI period of 2-4 days is
considered ideal for drought tolerance [65]. Results by several investigators [66-68] recorded negative correlations of ASI with plant yield and plant height. The study by Monneveux et al. [32] suggested that selection for heavier grains and smaller tassels may help to increase grain yield under water-limited environments in the near future.

The results of the present study clearly revealed significant phenotypic diversity of the Egyptian maize hybrids and populations. Furthermore, this diversity among maize hybrids and populations could be related to different plant response to different environments. Therefore, these promising maize hybrids and populations could be potentially utilized for the introgression of adaptive traits, which may be found in extreme environments [43]. The biplot method was first recommended by Yan et al. [38]. In the present study, the correlations between some plant characteristics and grain yield of maize genotypes was visually assessed. The biplot method allowed easy and better assessment of correlations between the traits in each environment.

5. CONCLUSION

The principal component analysis (PCA) of morphological data was able to assess genetic diversity and allowed efficient and reliable assessment of investigated traits of a collection of 19 maize Egyptian hybrids and populations under different stressed environments. Our data showed substantial variation in morphological traits among these genotypes. The GT-biplot method allowed easy and better assessment of correlations among the traits and to identify the reliable secondary traits for improving grain yield under drought and/or low N stressed conditions. It was observed that 100- kernel weight (100-KW), ears/plant (EPP), and anthesis-silking interval (ASI) were identified as the mostly correlated traits with grain yield in all environments. It was also concluded that high values of EPP, ear height, days to anthesis, days to silking, plant height, and chlorophyll concentration index (CCI) under drought stress, kernels/row (KPR), EPP, 100-KW, CCI, and grain starch content (GSC) under low N environment; and KPR, EPP and 100-KW and GSC under drought combined with low N environment and low values of ASI under the three stressed environments could be considered selection criteria for high grain yield under respective stressed environments or for drought and/or low N tolerance. In general, the study recommended selection for high values of KPR, EPP and 100-KW and low value of ASI in order to increase grain yield under drought and low N stressed conditions. Further investigations should be undertaken for collection, assessment and utilization of maize germplasm.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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