**ARTICLE**

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# Molecular markers and GGE biplot analysis for selecting higher-yield and drought-tolerant maize hybrids

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**Abstract**

Improving maize (*Zea mays* L.) genotypes for higher productivity and tolerance to drought stress depends mainly on physiological and molecular markers. Therefore, this study aims at breeding maize for drought tolerance and high potentiality by selection based on molecular markers, photosynthetic parameters; and easy graphic methods that help in selecting elite genotypes across diverse environments. An $8 \times 8$ half diallel analysis was used at two locations involving drought and normal irrigation treatments to study parental genetic diversity (GD) and combining ability (general combing ability [GCA] and specific combining ability [SCA]) in F1 of maize. Fingerprinting of parents was made using simple sequence repeat (SSR) markers. Fifty-eight alleles were ranged from two to five alleles per locus with an average of 0.63 alleles per locus. The average of polymorphic information content (PIC) was 0.63. Cuvette temperature (oc) was lowest by the cross L14 × L36. The cross L8 × L34 expresses the highest value for Quantum sensor (μmol m$^{-2}$ s$^{-1}$), net $CO_2$ assimilation rate and chlorophyll content. As for leaf diffusive resistance (LDR) four crosses exhibited significant desirable LDR values. Concerning rate of leaf transpiration (LTR) (μg cm$^{-2}$ S$^{-1}$) the cross (L5 × L104) gave the lowest value. Most hybrids exhibited desirable values for drought susceptibility index. For grain yield plant$^{-1}$, five F$_1$ crosses, that is, L5 × L34, L8 × L14, L8 × L14, L30 × L104, and L36 × L104 expressed the most desirable SCA effects. These crosses are promising in maize breeding programs. Based on GGE biplot analysis, genotype nos. 8 and 10 exhibited the highest grain yield plant$^{-1}$ and ranked the first across all environments.

## 1 | INTRODUCTION

Maize (*Zea mays* L.) is an important cereal and fodder crop cultivated in the world. It represents a source of nutrients as well as compounds such as phenolics, phytosterols, and carotenoids (Shah et al., 2016). Its annual total world production was around 1.148 Tg from197.2 Mha in 2019. In Egypt,

**Abbreviations:** AEC, average environment coordinate; DSI, drought sensitivity index; GCA, general combining ability; GD, genetic diversity; GGE, genotype x environment; LDR, leaf diffusive resistance; LTR, leaf transpiration rate; MET, multi-environment trial; PIC, polymorphic information content; RCBD, randomized complete block design; SCA, specific combining ability; SSR, simple sequence repeat; TL-TA, leaf-to- air temperature gradient

it is the second cereal crop after wheat (*Triticum aestivum* L.) with an area of 0.99 M ha in 2019 producing 7.49 Gg (FAOSTAT, 2020). Maize plants may suffer from various abiotic stresses such as salinity, drought, and high temperature at different stages of its growth (Sah et al., 2020).

Drought stress is a great obstacle in maize production where shortage of water supply reduces yield components, leaf photosynthesis, and transpiration in maize plants leading to yield reduction in maize (Jiang et al., 2018).

Different abiotic stresses include high temperature and water deficit accentuated by climate change and irrigation water shortage (Saxena et al., 2018).

Although, the present varieties have high yield productivity, they have not been fully explored especially for drought stress. Breeding programs can effectively solve this problem through developing new cultivars with high potentiality under drought stress conditions (Badr et al., 2020).

Grain yield of maize is a polygenic trait governed by interaction between genotype and environment (G × E) (Adu, Badu-Apraku, et al., 2019). Therefore, evaluating new maize genotypes under harsh environments is a priority to detect the most suitable genotypes. Determination of drought susceptibility index (DSI) is the main approach to distinguish drought-tolerant genotypes (Angearu et al., 2020). An alternative way to detect tolerant genotypes is selection based on physiological traits. These traits should show a high correlation with drought tolerance and yield potentiality (Boscaiu & Fita, 2020). Secondary traits (physiological and photosynthetic parameters) are used as indirect selection markers according to their role in photosynthesis(Anjum et al., 2011). The photosynthetic system is adversely affected by drought (Kapoor et al., 2020). Therefore, maize genotypes must be evaluated for these physiological traits aiming at enhancing stress tolerance (Ghatak et al., 2017). Some studies used a gas analysis system to measure net photosynthetic rate (PN) together with transpiration rate (E), and stomatal conductance (gs), as physiological markers for developing new drought-tolerant maize plants (Anjum et al., 2011; Sah et al., 2020; Sicher & Barnaby, 2012). Such efforts demonstrated that PN (or gs) correlated significantly with drought tolerance and can be used as a secondary selection marker for stress tolerance. Chlorophyll content is always a sufficient indicator for selecting maize genotypes against sensitivity to water deficit (Gitelson et al., 2014; Lin et al., 2015). The group of chemical parameters used as a marker for selection to drought tolerance include the simple sequence repeat (SSRs) is an important procedure to accelerate the genetic improvement in maize (Kamara et al., 2020). Yet, evaluating hybrid progeny especially for drought tolerance and yield potential requires investigating the parental inbred line to detect diversity and markers associated with stress tolerance (Nyaligwa, 2016).

Grain yield performance as a quantitatively inherited trait is often affected by environment causing significant

**Core Ideas**

- Five new maize hybrids with higher yield potentiality and tolerance to drought were developed.
- Crucial metabolic functions related to yield productivity of maize were identified.
- The polymorphic information content and gene diversity of 40 SSR markers associated with drought tolerance were investigated.

interactions between genotype and environment (G × E). Using the principal components model as multivariate analysis, graphic models have been extensively used including GGE biplot (Badu-Apraku & Akinwale, 2019; Fan et al., 2007). Such methods give a set of functional graphs that help plant breeders to explore the interrelationships among environments, tested genotypes, and associations between genotypes and environment (Malik et al., 2019).

The current study aims at screening parental material with SSRs markers and study the effect of some photosynthetic traits in maize crosses under water stress conditions. Also, yield stability of hybrids derived from diallel cross analysis along with a check hybrid will be studied in two locations under different water regimes in each location using GGE Biplot analysis.

## 2 | MATERIALS AND METHODS

To achieve the objective of this work, new maize hybrids were developed from maize genotypes originally obtained from two countries (Egypt and Mexico) and a modern protocol for evaluation, were used to identify and select elite hybrids under normal irrigation and drought conditions.

The previously mentioned protocol depends on screening maize hybrids based on SSR markers and studying photosynthetic and yield traits under various environments to detect their stability using GGE biplot analysis.

The methodology used in this experiment will help corn breeders in many regions of the world to evaluate and select elite maize hybrids under similar conditions of this work.

## 2.1 | Plant materials

Eight elite white maize lines varying in their yielding abilities and tolerance to drought stress were used in this study, that is, L5 (P1), L8 (P2), L30 (P4), L34 (P5), L36 (P6), L68 (P7), and L104 (P8) which were released more than 10 yr ago from SC 10, Pioneer 514, Cairo 1, Co. 108,

SC. 10, Giza 2, and Sabaeny (Egypt), respectively. Parent L14(P3) was imported from CYMMT (CYMMT Entry 9), Mexico. Single cross Hytech 2031 was used as a check hybrid.

## 2.2 | DNA extraction

Genomic DNA was extracted from eight inbred lines after 20 d from planting using the modified CTAB method (Doyle & Doyle, 1990). The integrity of DNA was verified on 1% agarose gel using gel electrophoresis image (Gel Doc. Bio-Rad). The DNA purity and concentration were measured at absorbance ratio of A260/A280 using BioTek Epoch2 microplate reader (Thermo Scientific).

## 2.3 | Polymerase chain reaction amplification and simple sequence repeat analysis

Eight genotypes were subjected to molecular diversity analysis using 40 maize SSR primers. Only 16 SSR markers were found to be polymorphic and they were used for the SSR analysis. The polymerase chain reaction (PCR) reaction was done according to Badr et al. (2019). The reaction was performed in the (AriaMx) PCR Thermo cycler consisting of 95 °C for 3 min followed by 35 cycles at 95 °C for 50 s, annealing temperature was calculated for each primer, then for 1 min with an extension of 72 °C for 1 min followed by final extension temperature at 72 °C for 5 min. The products of PCR were stored at −20 °C and verified on 2% agarose gel electrophoresis image using GeneRuler 1 kb DNA ladder, then visualized using gel documentation system (Gel Doc. BioRad).

## 2.4 | Genetic diversity

From the maize genetic and genomic database (GDB) (www.maizegdp.org) the SSR marker were determined to identify the polymorphic pairs. For SSR analysis 16 markers of polymorphic were used (Supplemental Table S1).

The PCR amplicons of polymorphic primers were scored as binary data matrix of 0, 1 for each marker based on absence and presence of target bands. The allele diversity of each marker was assessed through calculating the gene diversity, major allele frequency, the number of allele and polymorphic information content. According to Botstein et al. (1980), the value of polymorphic information content was calculated of each SSR marker. Genetic distances were calculated between pairs of inbred strains and phylogenetic tree associated with the neighborhood using the PAST program.

## 2.5 | Field trials

All diallel combinations without reciprocals were constituted from the eight parental inbred lines to produce 28 $F_1$ crosses in the first season of 2018. In the next season 2019, $F_1$ crosses and the check hybrid SC Hytech 2031 were evaluated at two locations (Agriculture Research and Experimental Station of the Faculty of Agriculture, Moshtohor and Agriculture Research Center, Giza, Egypt). In each location two independent trials were undertaken, by adding irrigation every 13 d (normal irrigation) and water-stress irrigation every 21 d (drought environment) using a randomized complete block design (RCBD) with three replications in each environment. Each hybrid was one ridge, 6 m long to get 30 plants. Plant-to-plant and ridge-to-ridge spacings were kept at 20 and 70 cm, respectively. Three kernels were planted $hill^{-1}$ on one side of the ridge then seedlings were later thinned to one plant $hill^{-1}$ to adjust the plant stand. The other cultural practices were followed properly as recommended for ordinary maize in the area (El-Hosary et al., 2019).

## 2.6 | Photosynthetic parameters

Carbon dioxide rates and water vapor exchange and diffusive conductance are substantial for consideration of water relatives and carbon (C) of plants. A portable porometer "steady-state porometer, LICOR, LI-1600, Lincoln, NE, USA" was designated for assessing the steady-state $CO_2$ and $H_2O$ exchange degrees of maize leaves.

The entire porometer comprises an open gas exchange that displays $CO_2$ and $H_2O$ incoming and exit a cuvette that is fixed on or around leaves (Agehara & Leskovar, 2012; Bunce, 1993). This device might also be utilized to calculate the response of $CO_2$ curves in the field. Leaf diffusive resistance (LDR) was determined according to Bunce (2006). Leaf transpiration rate (LTR) was recorded using the steady-state porometer on the same leaf. Leaf temperature was calculated by the thermocouple of the steady-state porometer pressed against the adaxial and abaxial surfaces of the leaf, and the leaf-to-air temperature gradient (TL-TA) was measured by using the atmospheric temperature. Genotypes arranged in RCBD were subjected to ANOVA according to Gomez and Gomez (1984) and Duncan's multiple range test (Duncan, 1955) at $P \leq .05$ was applied to make a comparison among means.

## 2.7 | Grain yield $plant^{-1}$

After harvest grain yield of maize plant was recorded from 10 guarded plants and adjusted to a uniform moisture basis of 15.5%. The data obtained were analyzed and subjected

**TABLE 1** Chromosome number, allele range and numbers, frequency of major allele, allele diversity, and polymorphic information content (PIC)

| Marker | Chromosome no. | Size range | Repeat type | Alleles no. | Frequency of major allele | Allele diversity | PIC |
|--------|---------------|------------|-------------|-------------|---------------------------|------------------|-----|
| | | bp | | | | | |
| bnlg1028 | 10 | 140–200 | AG (12) | 3 | 0.5 | 0.47 | 0.63 |
| bnlg 1083 | 1 | 140–182 | AG (29) | 4 | 0.37 | 0.38 | 0.64 |
| bnlg1166 | 6 | 136–145 | AG (14) | 2 | 0.63 | 0.38 | 0.55 |
| bnlg1241 | 4 | 140–260 | AG (21) | 4 | 0.5 | 0.47 | 0.66 |
| bnlg1866 | 1 | 90–130 | AG (11) | 4 | 0.38 | 0.5 | 0.72 |
| bnlg2190 | 10 | 180–220 | AG (31) | 5 | 0.38 | 0.47 | 0.75 |
| umc1542 | 2 | 144–168 | AG (10) | 3 | 0.37 | 0.38 | 0.66 |
| bnlg1082 | 9 | 267–278 | AG (11) | 2 | 0.62 | 0.38 | 0.47 |
| bnlg1297 | 2 | 100–170 | AG (32) | 3 | 0.5 | 0.5 | 0.63 |
| dupssr12 | 1 | 98–135 | AC (15) | 5 | 0.38 | 0.47 | 0.75 |
| bnlg1035 | 3 | 86–125 | AG (13) | 3 | 0.75 | 0.22 | 0.41 |
| bnlg1209 | 9 | 160–185 | AG (12) | 5 | 0.38 | 0.22 | 0.75 |
| umc1016 | 7 | 80–148 | CT (25) | 5 | 0.3 | 0.38 | 0.75 |
| nco12 | 6 | 100–120 | CT | 2 | 0.62 | 0.38 | 0.47 |
| bnlg2328 | 7 | 160–240 | AG (33) | 5 | 0.38 | 0.38 | 0.75 |
| bnlg1225 | 2 | 100–156 | AG (14) | 3 | 0.63 | 0.5 | 0.53 |
| Average | | | | 3.6 | 0.48 | 0.4 | 0.63 |

*Note.* PIC, polymorphic information content.

to genetical analysis of half diallel analysis as described by Griffing (1956). Drought susceptibility index (DSI) was calculated for grain yield plant$^{-1}$ using the following formula: $DSI = \frac{Ys}{Yp}$, where, $Ys$ = grain yield of genotype in stress environment and $Yp$ = yield of genotype in non-stress condition.

The GGE biplot analysis was proposed to analyze the multi-environment trial (MET) data using the graphical presentation. Although GGE biplot analysis generates graphics that allow visual presentation for MET data, the GGE biplot graphs are highly preferred because they are easy to construct, more effective, and more informative diagnostic for MET data (Yan et al., 2007; Menkir et al., 2004).

## 3 | RESULTS

### 3.1 | Reactions of simple sequence repeat polymorphisms, allele diversity, and analysis of cluster

In this study 40 SSR primer pairs were investigated, 16 pairs revealed polymorphic pattern among the eight inbred lines (Table 1 and Figure 1). A total number of 58 alleles were ranged from two to five alleles per locus with an average of 0.63 alleles per locus. The average of major allele frequency was 0.48 with a stretched range from 0.30 at locus

umc1016 to 0.75 at locus bnlg1035. The gene diversity values ranged from 0.22 at loci bnlg1035 and bnlg1209 to 0.50 at loci bnlg1225 and bnlg1866 with an average of 0.4. While, the average of polymorphic information content (PIC) was 0.63 with values varied from 0.41 at locus bnlg1035 to 0.75 at locus bnlg 2190. Genetic distance estimates based on SSR markers ranged from 0.12 to 0.94 with an average of 0.65. The lowest genetic distance (0.12) was obtained between the inbred lines (L30 and L104), whereas the highest genetic distance (0.94) was observed between the inbred lines (L14 and L68), (L14 and L30) (Table 2). The GD dendrogram revealed the close similarity among the inbred lines; L5, L34, L68 that clustered together in a main cluster. On another hand, L14 diverged in subcluster and the other lines L30, L104, L36, and L8 were clustered together (Figure 2).

### 3.2 | Photosynthetic parameters

Cuvette temperature (oc) show that the lowest value was obtained by the cross L14 × L36 and did not differ significantly from any of L5 × L34, L5 × L36, L8 × L30, L14 × L34, L30 × L34, L30 × L34 and L34 × L68 (Figure 3) and (Supplemental Table S2). Regarding Quantum sensor {μmol m$^{-2}$ s$^{-1}$}, Figure 4 show that cross L8 × L34 recorded highest value with no significant difference with any of L8 × L36, L8 × L68, L14 × L36 and L34 × L104. As for leaf diffusive
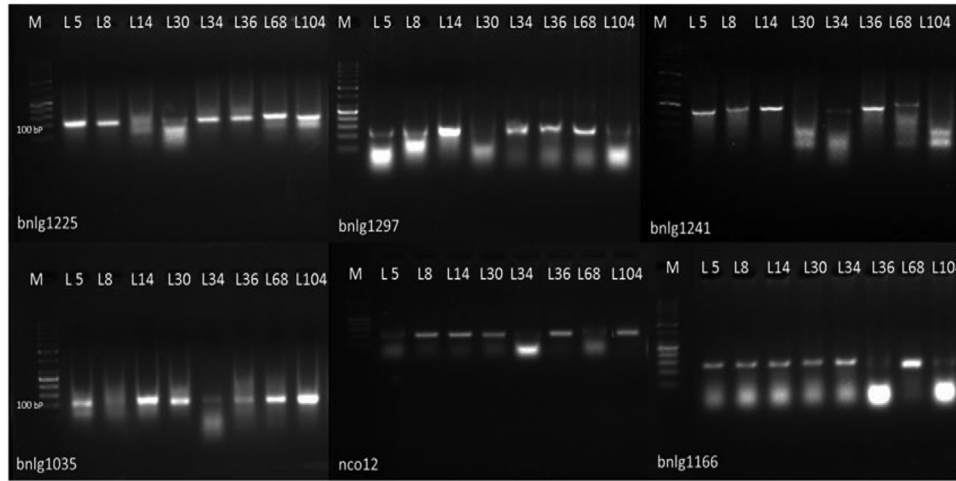
**FIGURE 1** Polymerase chain reaction amplicons of some simple sequence repeat markers with maize lines. M denotes 100 bp DNA ladder

**TABLE 2** Matrix of genetic distance, constructed from simple sequence repeat data, for the eight lines of maize

| Parent | L5 | L8 | L14 | L30 | L34 | L36 | L68 | L104 |
|---|---|---|---|---|---|---|---|---|
| L5 | _ | 0.643 | 0.727 | 0.733 | 0.385 | 0.692 | 0.500 | 0.714 |
| L8 | | – | 0.910 | 0.667 | 0.714 | 0.250 | 0.714 | 0.636 |
| L14 | | | _ | 0.931 | 0.700 | 0.875 | 0.940 | 0.929 |
| L30 | | | | _ | 0.615 | 0.600 | 0.744 | 0.125 |
| L34 | | | | | _ | 0.769 | 0.462 | 0.692 |
| L36 | | | | | | _ | 0.769 | 0.556 |
| L68 | | | | | | | _ | 0.583 |
| L104 | | | | | | | | _ |

resistance (LDR), Figure 5 shows that crosses L8 × L4, L8 × L36, L8 × L68, and L14 × L30 exhibited significant desirable LDR values.

Concerning rate of leaf transpiration (LTR) {µg cm$^{-2}$ S$^{-1}$} the cross (L5 × L104) gave the lowest value with no significant differences with any of L5 × L30, L5 × L36, L5 × L68, L8 × L14 and L8 × L68 (Figure 6). The check hybrid Hytech
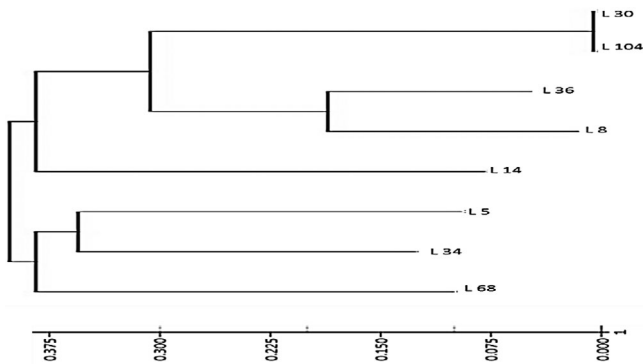


**FIGURE 2** Neighbor joining phylogenetic tree of the eight maize lines using PAST
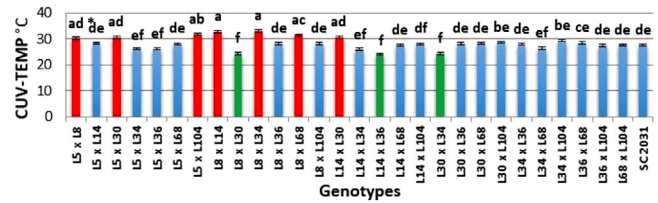


**FIGURE 3** Effect of drought stress on the behavior of all genotypes for cuvette temperature. *Means with the same letter are not different significantly at .05 level of probability, based on Duncan's multiple range test (Duncan, 1955)



**FIGURE 4** Effect of drought stress on the behavior of all genotypes for Quantum sensor (µmol m$^{-2}$ s$^{-1}$). *Means with the same letter are not different significantly at .05 level of probability, based on Duncan's multiple range test (Duncan, 1955)



**FIGURE 5** Effect of drought stress on the behavior of all genotypes for leaf diffusion resistance. *Means with the same letter are not different significantly at .05 level of probability, based on Duncan's multiple range test (Duncan, 1955)

**FIGURE 6** Effect of drought stress on the behavior of all genotypes for leaf transpiration rate. *Means with the same letter are not different significantly at .05 level of probability, based on Duncan's multiple range test (Duncan, 1955)
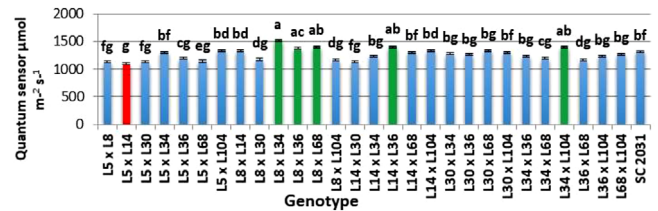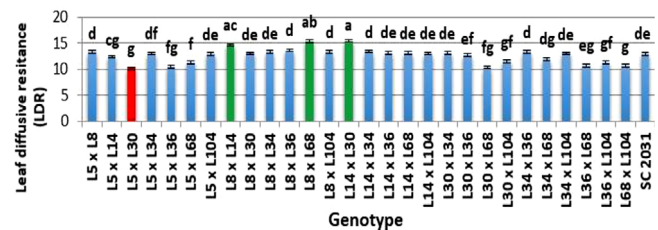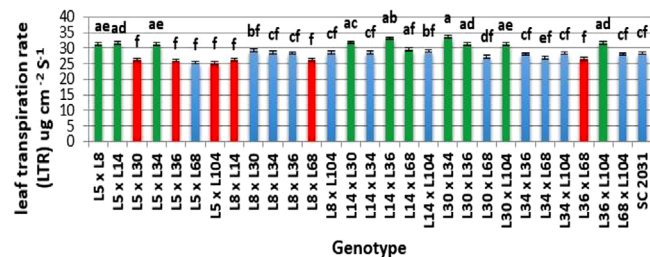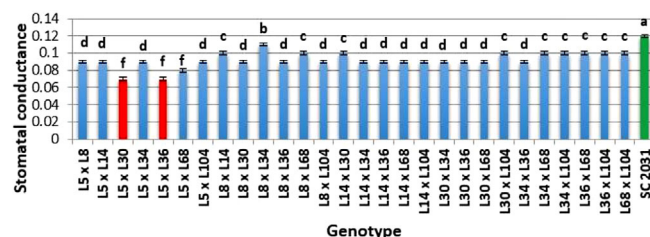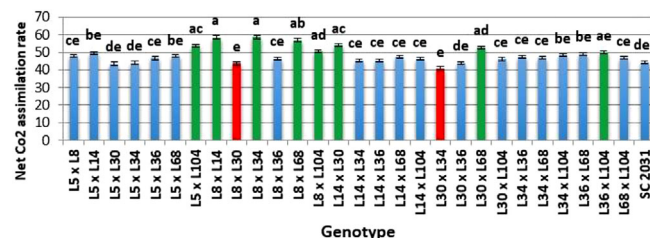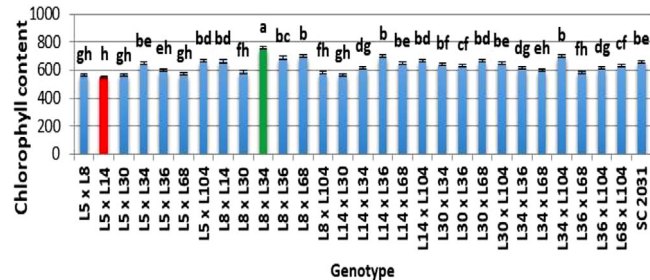


**FIGURE 7** Effect of drought stress on the behavior of all genotypes for stomatal conductance. *Means with the same letter are not different significantly at .05 level of probability, based on Duncan's multiple range test (Duncan, 1955)

2031 exhibited the highest and significant value for stomatal conductance (Figure 7). For Net $CO_2$ assimilation rate, the maximum mean value was recorded with the cross L8 × L34 (Figure 8). However, the crosses L5 × L104, L8 × L14, L8 × L68, L14 × L30, and L30×L68 did not differ significantly from the check hybrid. As for Chlorophyll content), the cross L8×L34 exhibited the highest significant value for this trait followed by the cross L34 × L104 and L8 × L68 (Figure 9).



**FIGURE 8** Effect of drought stress on the behavior of all genotypes for net $CO_2$ assimilation rate. *Means with the same letter are not different significantly at .05 level of probability, based on Duncan's multiple range test (Duncan, 1955)



**FIGURE 9** Effect of drought stress on the behavior of all genotypes for chlorophyll content. *Means with the same letter are not different significantly at .05 level of probability, based on Duncan's multiple range test (Duncan, 1955)

**TABLE 3** Variance analysis for grain yield plant$^{-1}$ at each environment at the two locations

| SOV | df | Moshtohor | | Giza | |
|---|---|---|---|---|---|
| | | Normal | Stress | Normal | Stress |
| Replication | 2 | 6.04 | 26.80 | 1,910.95** | 764.67** |
| Genotype | 27 | 2,088.66** | 909.35** | 1,987.11** | 1,523.3** |
| Error | 54 | 25.36 | 35.46 | 28.87 | 37.25 |
| GCA | 7 | 670.37** | 284.17** | 429.23** | 936.86** |
| SCA | 20 | 705.27** | 309.75** | 743.97** | 357.79** |
| Error | 54 | 8.45 | 11.82 | 9.62 | 12.42 |
| GCA/SCA | | 0.95 | 0.92 | 0.58 | 2.66 |

*Note.* SOV, source of variance; GCA, general combining ability; SCA, specific combining ability.

**Highly significant mean squares at .01 level of probability.

## 3.3 | ANOVA

Variance analysis for crosses and the combining abilities of each studied environment for maize grain yield are illustrated in Table 3. Results revealed that mean squares due to hybrids were significant for this trait in each studied environment. The variance associated with both types of combining ability (Table 3), that is, general and specific was significant for grain yield plant$^{-1}$ in each environment. Mean squares of specific combining ability (SCA) surpassed those of general combining ability (GCA) in both environments at Moshtohor location and normal environment at Giza location. However mean squares of GCA exceeded those of SCA in a stress environment at Giza location.

## 3.4 | Mean performance, drought sensitivity index, and superiority relative to check hybrid

Regarding the mean performance of grain yield plant$^{-1}$, four F$_1$ crosses, namely, L5 × L104, L8 × L14, L8 × L34, and L14 × L30 in normal environment and L5 × L104, L8 × L14, L8

**TABLE 4** Mean values of maize crosses and check hybrid SC hytech 2031 for grain yield plant$^{-1}$ at each environment and estimate of drought sensitivity index (DSI) in each location

| Code | Cross | Moshtohor | | | Giza | | | Mean across environment |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | Normal | Stress | DSI | Normal | Stress | DSI | |
| G1 | L5 × L8 | 184.00 | 134.33 | 0.73 | 188.63 | 142.80 | 0.76 | 162.44 |
| G2 | L5 × L14 | 188.00 | 138.00 | 0.73 | 159.57 | 109.06 | 0.68 | 148.66 |
| G3 | L5 × L30 | 170.33 | 118.00 | 0.69 | 161.27 | 129.16 | 0.80 | 144.69 |
| G4 | L5 × L34 | 210.00 | 151.67 | 0.72 | 199.60 | 153.22 | 0.77 | 178.62 |
| G5 | L5 × L36 | 169.00 | 105.00 | 0.62 | 186.11 | 125.68 | 0.68 | 146.45 |
| G6 | L5 × L68 | 168.33 | 138.67 | 0.82 | 169.63 | 134.74 | 0.79 | 152.84 |
| G7 | L5 × L104 | 215.00 | 170.00 | 0.79 | 144.33 | 113.58 | 0.79 | 160.73 |
| G8 | L8 × L14 | 210.67 | 170.67 | 0.81 | 218.76 | 173.12 | 0.79 | 193.30 |
| G9 | L8 × L30 | 185.00 | 107.33 | 0.58 | 195.71 | 147.10 | 0.75 | 158.79 |
| G10 | L8 × L34 | 223.33 | 152.33 | 0.68 | 234.01 | 158.39 | 0.68 | 192.02 |
| G11 | L8 × L36 | 150.33 | 135.67 | 0.90 | 186.30 | 135.45 | 0.73 | 151.94 |
| G12 | L8 × L68 | 207.67 | 153.33 | 0.74 | 213.80 | 168.50 | 0.79 | 185.82 |
| G13 | L8 × L104 | 153.00 | 121.33 | 0.79 | 119.48 | 104.13 | 0.87 | 124.49 |
| G14 | L14 × L30 | 220.00 | 122.67 | 0.56 | 223.42 | 155.39 | 0.70 | 180.37 |
| G15 | L14 × L34 | 142.67 | 139.00 | 0.97 | 177.55 | 137.68 | 0.78 | 149.22 |
| G16 | L14 × L36 | 154.67 | 135.33 | 0.87 | 151.65 | 114.31 | 0.75 | 138.99 |
| G17 | L14 × L68 | 165.00 | 120.00 | 0.73 | 174.21 | 136.41 | 0.78 | 148.90 |
| G18 | L14 × L104 | 137.67 | 123.33 | 0.90 | 162.66 | 130.33 | 0.80 | 138.50 |
| G19 | L30 × L34 | 144.67 | 101.67 | 0.70 | 169.47 | 127.42 | 0.75 | 135.81 |
| G20 | L30 × L36 | 150.00 | 132.67 | 0.88 | 157.10 | 121.16 | 0.77 | 140.23 |
| G21 | L30 × L68 | 147.33 | 131.33 | 0.89 | 142.30 | 111.58 | 0.78 | 133.13 |
| G22 | L30 × L104 | 194.00 | 151.00 | 0.78 | 199.33 | 166.56 | 0.84 | 177.72 |
| G23 | L34 × L36 | 152.67 | 119.67 | 0.78 | 162.93 | 104.99 | 0.64 | 135.06 |
| G24 | L34 × L68 | 186.67 | 149.67 | 0.80 | 166.48 | 119.69 | 0.72 | 155.63 |
| G25 | L34 × L104 | 183.33 | 137.00 | 0.75 | 138.35 | 104.88 | 0.76 | 140.89 |
| G26 | L36 × L68 | 143.33 | 118.67 | 0.83 | 95.96 | 82.64 | 0.86 | 110.15 |
| G27 | L36 × L104 | 197.33 | 135.33 | 0.69 | 135.91 | 106.88 | 0.79 | 143.86 |
| G28 | L68 × L104 | 169.67 | 136.67 | 0.81 | 165.44 | 114.74 | 0.69 | 146.63 |
| G29 | SC 2031 | 202.33 | 143.67 | 0.71 | 208.17 | 149.41 | 0.72 | 175.89 |
| LSD .05 | | 7.28 | 7.44 | 0.14 | 8.73 | 9.91 | 0.10 | |
| Mean environment | | 176.76 | 134.28 | | 172.69 | 130.31 | | |

× L34 and L8 × L68 in stress condition at Moshtohor location exhibited the best significant mean values when compared with Hytech 2031. For the Giza location, the superior hybrids for grain yield plant$^{-1}$ were L8 × L14, L8 × L34, and L14 × L30 in normal condition and L8 × L14 and L8 × L68 under stress condition with significant superiority as compared to Hytech 2031.

For screening maize hybrids according to their sensitivity for drought, the index DSI was used as an appropriate parameter to do this task. Grain yield plant$^{-1}$ across genotypes exhibited significant differences between stress and normal environments at each location. The differences varied among maize genotypes (Table 4). The highest DSI for grain yield was given by L8 × L36, L14 × L634 L14 × L104, L30 × L36, and L30 × L68 at Moshtohor location and L8 × L104, L30 × L104, and L36 × L68 at the Giza location (Table 4, Figure 10).

Concerning relative superiority of grain yield plant$^{-1}$ over check hybrid (Table 5), the F$_1$ crosses L5 × L14, L5 × L104, L8 × L14, L8 × L34, and L8 × L68 in the studied two environments at Moshtohor location. Also, the crosses L8 × L14 and L8 × L34 in both environments at the Giza location showed significant and desirable relative superiority over check hybrid. The useful superiority of the mentioned crosses ranged between 3.79 and 18.79%.
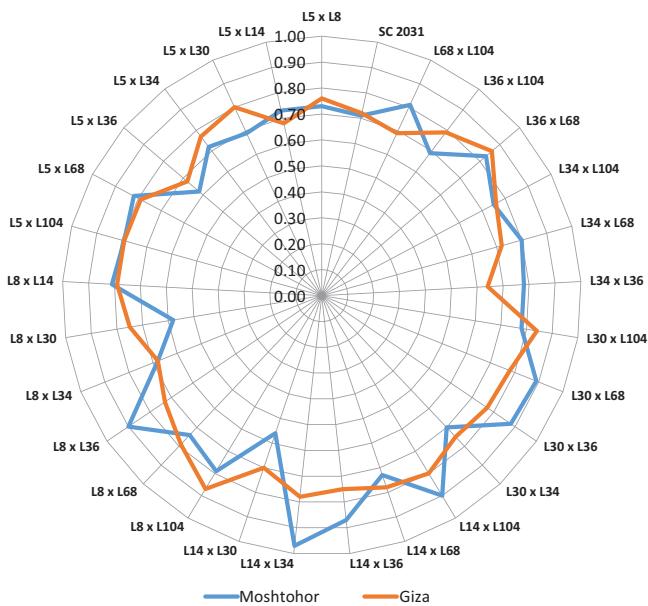
**FIGURE 10** Drought sensitivity index for all studied crosses at each location

## 3.5 | Combining ability effects

On basis of GCA effect $\hat{g}_i$ analysis (Figure 11) for studied parents inbred lines, L5 and L8 in Moshtohor location and L8, L14, L30, and L34 in the Giza location exhibited good general combiners for grain yield plant$^{-1}$. Furthermore, the parental inbred line L8 expressed the best general combiner for the mentioned trait at both locations. However, the other parents showed undesirable $\hat{g}_i$ effects for this trait. Positive and significant SCA effects $\hat{S}_{ij}$ for this trait were revealed by four F$_1$ crosses, that is, L5 × L34, L8 × L14, L8 × L14, L30 × L104, and L36 × L104, while, the other desirable interactions of $\hat{S}_{ij}$ were detected by 4, 6, 5, and 4 crosses at normal environment in Mostohor (normal), Mostohor (stress), Giza (normal) and (stress), respectively (Table 5). However, the best SCA effects for grain yield of maize were detected in the crosses L36 × L104 (37.24$^{**}$) under normal conditions and L8 × L14 (28.59$^{**}$) under stress conditions at Moshtohor location. At Giza location the cross L30 × L104 gave the most desirable SCA under normal and stress conditions being 42.21$^{**}$ and 39.49$^{**}$ (** means highly significant), respectively. The aforementioned crosses contain good combiner parents or involving good × good general combiners. The other crosses had either significant positive or insignificant $\hat{S}_{ij}$ effects.

## 3.6 | GGE-biplot analysis

### 3.6.1 | Yield and stability of studied genotypes

It is obvious that the GGE-biplot model accounts for 92.00% of the total variance representing 73.89 and 18.11% variance

attributable to the first (PC1) and second (PC2) principal component respectively (Figure 12).

Ten genotypes, that is, nos. 1, 9, 22, 26, 4, 14, 12, 10, 8, and 11 are located on the right of the original points. Genotype nos. 8 and 10 exhibited the highest grain yield plant$^{-1}$ and ranked the first across all environments (Table 4, Figure 12). Theses genotypes recorded the highest average grain yield (large PC1 scores), but the remaining genotypes were below average (PC1 scores <0) (Table 4). The responses of genotypes located at the left of the origin point were less than those of other positions.

## 3.7 | Ideal genotype analysis

As shown in Figure 13, the percentage of the total variance that is explained by the components (PC1 and PC2) was 92.00% indicating goodness of fit and validity of the GGE biplot method. The straight and single-arrow line (abscissa) passes through the biplot origin is referred to as average Environment Coordinate (AEC). The direction of the arrow determines the higher mean performance for genotypes. The small circle spotted on this line represents the average of environment PC1 and PC2 scores. It is defined by the average coordinates of all tested environments. However, the line (ordinate) passes through the origin of biplot and is perpendicular to the AEC line indicates the stability of the genotype. Thus, the genotype located closer to the AEC line in the two directions had more stable yield and vice versa.

Consequently, genotypes with above-average mean are descending and ranked as follows: G8 > G10 > G14 > G12 > G28 > G26 > G4 > G9 > G1 > G7 > G24. The remaining genotypes had below-average mean yield (Figure 8). Concerning the stable genotypes of G8, G10, G14, G12, G28, G26, G4, G9, and G1 which had high yield and located very close to the AEC line were reflecting their above average stability while genotypes G7 and G14 showed below average stability because they were slightly placed away from AEC abscissa.

## 3.8 | Correlation between genetic distance based on simple sequence repeat marker and F1 hybrid performance in each environment

The association between GD and each of mean value of grain yield plant$^{-1}$ which calculated for 28 cross combinations had insignificant or weak correlation $r = -.01, -.06, .11, .15$ for Moshtohor normal environment, Moshtohor stress environment, Giza normal environment and Giza stress environment, respectively. Based on GGE biplot analysis 10 genotypes, that is, genotype nos. 1, 4, 8, 9, 10, 11, 12, 14, 22, and 26 were highly yielded across environments.

**TABLE 5** Estimation of specific combining ability (SCA) effects and relative superiority over check hybrid SC 2031 for grain yield plant$^{-1}$ at each environment

| | SCA effects | | | | Superiority relative to the check | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Moshtohor | | Giza | | Moshtohor | | Giza | |
| Cross | Normal | Stress | Normal | Stress | Normal | Stress | Normal | Stress |
| L5 × L8 | −17.98** | −8.86** | −10.44** | −7.32* | −9.06** | −6.5* | −9.38** | −4.42 |
| L5 × L14 | 1.9 | −0.86 | −24.69** | −28.86** | −7.08** | −3.94 | −23.34** | −27** |
| L5 × L30 | −14.54** | −6.8** | −19.79** | −9.11** | −15.82** | −17.87** | −22.53** | −13.55** |
| L5 × L34 | 19.79** | 12.48** | 18.58** | 23.64** | 3.79* | 5.57* | −4.11 | 2.56 |
| L5 × L36 | −0.21 | −22.75** | 33.83** | 15.29** | −16.47** | −26.91** | −10.6** | −15.88** |
| L5 × L68 | −12.65** | −0.08 | 8.7** | 11.49** | −16.8** | −3.48 | −18.51** | −9.82** |
| L5 × L104 | 23.68** | 26.87** | −6.2* | −5.14 | 6.26** | 18.33** | −30.66** | −23.98** |
| L8 × L14 | 23.02** | 28.59** | 9.91** | 14.99** | 6.12* | 18.79** | 5.09* | 15.87** |
| L8 × L30 | −1.43 | −20.69** | −9.94** | −11.37** | −8.57** | −25.29** | −5.99** | −1.54 |
| L8 × L34 | 31.57** | 9.92** | 28.39** | 8.6** | 10.38** | 6.03* | 12.41** | 6.02* |
| L8 × L36 | −20.43** | 4.7* | 9.43** | 4.85 | −25.7** | −5.57* | −10.51** | −9.34** |
| L8 × L68 | 25.13** | 11.37** | 28.28** | 25.04** | 4.64* | 6.73* | 2.71 | 12.78** |
| L8 × L104 | −39.87** | −25.02** | −55.64** | −34.8** | −24.38** | −15.55** | −42.6** | −30.3** |
| L14 × L30 | 49.46** | −1.02 | 32.59** | 9.12** | 8.73** | −14.62** | 7.33** | 4.01 |
| L14 × L34 | −33.21** | 0.92 | −13.25** | 0.09 | −29.49** | −3.25 | −14.71** | −7.85* |
| L14 × L36 | −0.2 | 8.7** | −10.41** | −4.09 | −23.56** | −5.8* | −27.15** | −23.49** |
| L14 × L68 | −1.65 | −17.63** | 3.5 | 5.15 | −18.45** | −16.47** | −16.31** | −8.7* |
| L14 × L104 | −39.32** | −18.69** | 2.34 | 3.6 | −31.96** | −14.15** | −21.86** | −12.77** |
| L30 × L34 | −29.98** | −22.36** | −18.13** | −10.51** | −28.5** | −29.23** | −18.59** | −14.71** |
| L30 × L36 | −3.65 | 20.09** | −1.75 | 2.42 | −25.86** | −7.66** | −24.53** | −18.9** |
| L30 × L68 | −18.1** | 7.75** | −25.2** | −20.03** | −27.18** | −8.58** | −31.64** | −25.32** |
| L30 × L104 | 18.24** | 23.03** | 42.21** | 39.49** | −4.12* | 5.1 | −4.25* | 11.48** |
| L34 × L36 | −6.32** | −7.3** | 4.11 | −5.07 | −24.55** | −16.71** | −21.73** | −29.73** |
| L34 × L68 | 15.91** | 11.7** | −0.98 | −3.23 | −7.74** | 4.18 | −20.02** | −19.89** |
| L34 × L104 | 2.24 | −5.36* | −18.73** | −13.51** | −9.39** | −4.64 | −33.54** | −29.8** |
| L36 × L68 | −6.43** | −7.86** | −42.76** | −21.09** | −29.16** | −17.4** | −53.9** | −44.69** |
| L36 × L104 | 37.24** | 4.47* | 7.57** | 7.69* | −2.47 | −5.8* | −34.71** | −28.46** |
| L68 × L104 | −2.21 | −5.25* | 28.46** | 2.68 | −16.14** | −4.87 | −20.52** | −23.2** |
| LSD 0.05 (sij) | 4.35 | 4.45 | 5.21 | 5.92 | | | | |
| LSD 0.01 (sij) | 5.77 | 5.90 | 6.92 | 7.86 | | | | |
| LSD 0.05 (sij-sik) | 6.65 | 6.79 | 7.97 | 9.05 | | | | |
| LSD 0.001 (sij-sik) | 8.81 | 9.01 | 10.56 | 12.00 | | | | |
| LSD 0.05 (sij-ski) | 5.94 | 6.08 | 7.12 | 8.09 | | | | |
| LSD 0.01 (sij-ski) | 7.88 | 8.06 | 9.45 | 10.73 | | | | |

The correlation between the mentioned crosses and GD were moderate $r$ = .29, .53, .31, and .45 for Moshtohor normal environment, Moshtohor stress environment, Giza normal environment, and Giza stress environment, respectively.

## 4 | DISCUSSION

The methodology approach of this work depends upon a protocol including several methods: (a) SSR analysis to detect dissimilarity among maize genetic resources which were
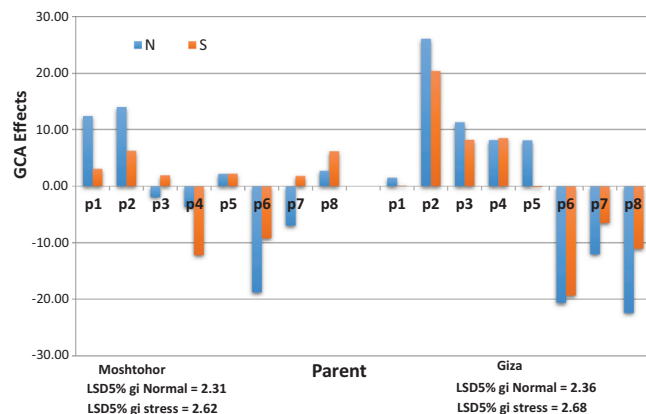
**FIGURE 11** Effects of general combining ability of the studied eight lines for maize grain yield plant⁻¹



**FIGURE 12** GGE-biplot polygon view for the which–one–where pattern for genotype scores and environments



**FIGURE 13** The view of mean value vs. stability of the GGE biplot

developed in two countries, (b) estimation of some photosynthetic traits which were related to yield potentiality of maize crosses, (c) detect mean performance and nature of gene action responsible for grain yield plant⁻¹ under normal irrigation and drought stress condition, and (d) study stability of maize crosses over various environments using GGE biplot analysis. This protocol will help breeders to detect the most desirable maize genotypes and how to use these genetic resources in different regions in the world with similar environmental conditions. Therefore, the results of this work will be discussed as follows:

## 4.1 | Simple sequence repeat analysis and allele diversity

In this study, the mean number of alleles per locus (3.6) was similar to other reports of Kamara et al. (2020), Steinfeld et al. (2015), and Menkir et al. (2004) which detected averages of 3.0, 2.9, and 2.7 alleles per locus, respectively. However, Adu, Awuku, et al. (2019) and Oppong et al. (2014) investigated genetic diversity in maize lines using SSR markers and reported relatively higher values of 5.7 and 6.21 alleles per locus, respectively. The varied number of alleles among different studies could be due to variations among genotypes, repeat length, population size, and the number of the SSR markers (Akinwale et al., 2014). The relatively high values observed in this study compared with other studies could be attributed to the high number of SSR markers (6) compared with a higher number (10) in a study by Kamara et al. (2020).

On another hand, in the current study lower allele number of 58 was recorded as compared with other studies (Adu, Awuku, et al., 2019; Vega-Alvarez et al., 2017) who detected 288 alleles and 649 alleles, respectively. Legesse et al. (2007) investigated lower allele number of 14 alleles. The differences in the number of alleles between the present study and the oth-
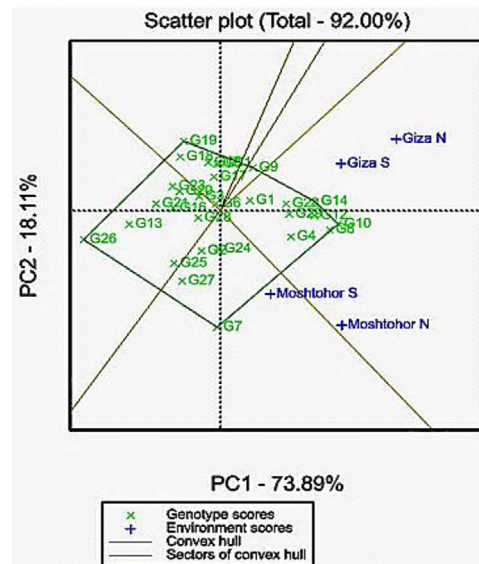
ers could be attributed to the genotypes or the approaches used for detection of polymorphic markers.
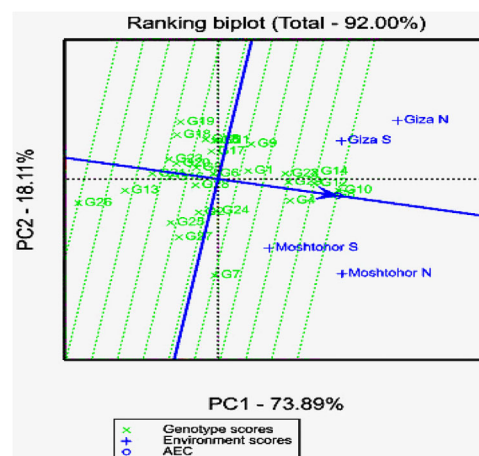
The obtained average gene diversity (0.50) demonstrates high polymorphism which agree with previous works (Kamara et al., 2020; Adu, Awuku, et al., 2019). The major alleles frequency exhibited an average of 0.48, demonstrating that 48.0% of the studied genotypes were having in common with all loci.

The polymorphic information contents (PIC) indicate SSR loci responsible for differences between genotypes (Legesse et al., 2007). The average PIC was 0.63 which is highly informative according to Botstein et al. (1980) who classified PIC values to three categories of information high (PIC > 0.5), reasonable (0.5 < PIC < 0.25), and slight (PIC < 0.25). Hence,

the markers proved of high discriminatory power as several loci showed PIC values >0.50.

## 4.2 | Photosynthetic parameters

Stomata is important in controlling gas exchange, mainly water vapor and $CO_2$, between the atmosphere and maize plants (Hetherington & Woodward, 2003). Exposure to moisture stress has negatively affected physiological processes in leaves and therefore changes in stomatal and thermal parameters correlated with the shortage of water especially in critical periods have to be interpreted by explanation (Mishra & Cherkauer, 2010). In this study leaf physiological traits can be divided into two groups the first involved Cuvette temperature c and leaf transpiration rate ($\mu g\ cm^{-2}\ s^{-1}$) (LTR) and the second involved Leaf diffusive resistance (LDR), Quantum sensor ($\mu mol\ m^{-2}\ s^{-1}$) stomatal conductance, Net $CO_2$ assimilation rate and chlorophyll content (Table 3, Figures 3–9). All such determinations gave a similar trend to how well plants can resist moisture stress. The behavior of the studied hybrids differed in response to stress, and this was reflected in significant differences between the studied genotypes. The adapted maize plants are not dependent on temperature adaptation and the ability of the plant to co-exist under stress conditions (Zhao et al., 2015).

Five hybrids limited their transpiration under drought, reflecting the importance of breeding (Leakey et al., 2006). Turgor-operated cells control diffusive resistance in cells, and water vapor as well as $CO_2$ and resistance of stomata. Maize dry matter production relates to radiation. Quantum sensors indicate maize efficiency. High recorded Quantum sensor indicates healthy growth and enhanced productivity under drought.

Canopy senescence accelerates under drought condition (Wolfe et al., 1988). Maize crosses which are drought resistant reduce leaf stomatal conductance as a result of water stress (Ray & Sinclair, 1997). C4 plants which avoid photorespiration, are saturated with $CO_2$. Improvements can be obtained under high presence of $CO_2$ when low conductance of stomata conserve moisture (Ghannoum et al., 2000). In the presence of high $CO_2$, gs of C4-plant decreases (Ainsworth & Long, 2005; Gáborčík, 2003; Gitelson et al., 2014; Meinzer et al., 1997; Uddling et al., 2007). Campbell et al. (1991) noted linear relationship between SPAD and chlorophyll.

## 4.3 | ANOVA

Mean squares due to crosses were significant for grain yield plant$^{-1}$ in each environment (Table 4) indicating high variation among maize crosses and their parental inbred lines. Significance of both types of combining ability mean squares

indicate that grain yield plant$^{-1}$ was governed by additive and non-additive genetic effects. The ration of GCA/SCA is used to identify the nature of inheritance of grain yield, and in the current study the ratio was less than unity for yield. Therefore, hybrid breeding can be effective for improvement of grain yield. This finding agrees with those of others (Abd El-Aty et al., 2019; Makumbi et al., 2011). On the other hand, high ratio was detected for grain yield plant$^{-1}$ in stress environment at Giza indicating predominance of additive genetic variance in the inheritance of this trait. These results agree with other results (Mason & Zuber, 1976; Al-Naggar & Atta, 2017). In general, the largest heterotic magnitude for this trait as the deviation of F1 mean performance from the check hybrid may strengthen the conclusion on the importance of non-additive gene action in controlling grain yield particularly at Moshtohor.

## 4.4 | Mean performance and drought sensitivity index

Generally, the highest grain yield plant$^{-1}$ was detected under normal irrigation treatment in both locations comparing with drought conditions. Water stress affects 45% of the world crops. It is a major constraint in maize production and the most negative factor causing yield reduction in semiarid regions (Sicher & Barnaby, 2012). Maize growth and yield affected by soil moisture regime caused yield deterioration, especially if water deficit occurs during the flowering or reproductive phase (Sah et al., 2020). Results of the current study show that the two single crosses, that is, L8 × L34 and L14 × L30 were the most effective among the studied crosses since they expressed the highest mean values under normal irrigation at Moshtohor and Giza location. Besides most desirable cross was L8 × L14 since it gave the highest grain yield plant$^{-1}$ under stress condition at both locations followed by the cross L5 × L104 at Moshtohor and L8 × L68 at Giza location.

Drought sensitivity index for maize genotypes under study ranged from 0.58 to 0.97 at Moshtohor and from 0.64 to 0.87 at Giza. More than 25% of studied genotypes exhibited high and stable yield from one environment to another. The useful superiority in yield over SC Hytech 2031 ranged from 13.79 to 15.78%. These crosses which have useful superiority gave higher susceptibility index. Hence it could be concluded that these crosses offer the possibility for increasing maize grain yield under drought stress.

Drought avoidance includes mechanisms which reduce water loss from plant or enhance water uptake. Therefore, a main objective in maize plant breeding programs is improving drought resistant genotypes. The ideal maize genotype must be a high yielder. However, under different environments most genotypes may differ by environmental changes.

## 4.5 | Combining ability effects

In general, mentioned combinations of desirable SCA effects especially in $F_1$ generation included good GCA combiner for both parents, they could be exploited for breeding promising varieties. Nevertheless, if the parental combination showed desirable high ŝij good combiner, such combinations would throw out desirable transgressive segregates provided that additive gene effect exist within the good combiner (as well as complementary and epistatic effects in the crosses) to reduce undesirable characteristics and maximize the character under consideration.

## 4.6 | GGE-biplot analysis

### 4.6.1 | Genotype stability and yield

GGE biplot was used to draw the polygon for interaction between Genotypes and Environments (G × E) giving various interpretations. The polygon emerged due to genotype markers farther away from the biplot origin so that other genotypes exist within the polygon. A biplot polygon view is the most appropriate to visualize the interaction patterns of environment/genotype and interpret the biplot. Furthermore 92.00% of the total variation divided into 73.89 and 18.11% variance attributable to PC1 and PC2 principal component respectively. This means the validity of GGE biplot analysis to differentiate among genotypes and environments. The genotypes which proved to be above average across environments were located on the right of original points.

The greater distance from the point of origin, points to the higher average performance of these genotypes. Thus, the two crosses 8 and 10 were stable and ranked first. However, genotypes located at the left of the plot origin were low values in performance and unstable genotypes.

The GGE biplot analysis compares the test genotypes to a reference thus specifying the position of an ''ideal'' genotype of highest average. This expresses no G × E interaction. Ideal genotype is based on the most stable genotype with highest yield. Such genotype is the one with the highest length on the average vector.

## 4.7 | Ideal genotype analysis

Ranking of the genotype is indicated by the GGE biplot. Thus, all genotypes ranked differently based on their stability and mean performance. The current results agree with those obtained by El Hosary (2020) and Yan (2010). The GGE biplot graph is often clear and easy to understand when few genotypes and environments are used, while if many genotypes and environments are used, the graph become so crowded that it gets difficult to visualize and interpret.

## 4.8 | Correlation between genetic distance based on simple sequence repeat marker and F1 hybrid performance in each environment

A low GD/yield correlation was detected for all genotypes pointing to a relating the two. However, it was observed that the correlation coefficient increased depending on the results of GGE biplot analysis. These results indicate the validity of SSR marker to detect the diversity among parental inbred line. These findings agree with those by El-Hosary and Elgammaal (2013) and Lanza et al. (1997)

## 5 | CONCLUSIONS

Eight parental inbred lines were tested using SSR markers. Then, 28 $F_1$ hybrids which producing from an 8 × 8 half diallel scheme crosses along with check hybrid SC Hyteck 2031 were evaluated at four diverse environments. The study aimed at studying genetic diversity (GD), mean performance, photosynthetic parameters, combining ability, and drought susceptibility index across different irrigation treatments. Forty SSR primer pairs were investigated; 16 pairs revealed polymorphic pattern among the eight inbred lines. Major allele frequency varied from 0.30 to 0.75 at loci umc1016 and bnlg1035, respectively, under overall average of 0.48. The gene diversity values ranged from 0.22 to 0.50 averaging 0.4. The variance associated with both types of combining ability was significant for grain yield plant$^{-1}$ in each environment. The SCA mean squares were higher than those of GCA in most cases. The parental inbred lines, L5, L8, L14, L30, and L34 exhibited good general combiners for grain yield plant$^{-1}$. Furthermore, the parental line L8 expressed the best general combiner for grain yield plant$^{-1}$. Positive and significant SCA effects for this trait were revealed by five $F_1$ crosses, that is, L5 × L34, L8 × L14, L8 × L14, L30 × L104 and L36 × L104. However, the best significant and desirable SCA effects for grain yield plant$^{-1}$ were detected in the crosses L36 × L104, L8 × L14 and L30 × L104. Based on GGE biplot analysis, 10 genotypes, that is, genotype nos. 1, 4, 8, 9, 10, 11, 12, 14, 22, and 26 had higher potentiality across environments.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## SUPPORTING INFORMATION

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