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## Assessment of Genetic Parameters and Drought Tolerance Indices in Maize Diallel Crosses.

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### ABSTRACT

A half diallel analysis among seven imported maize inbred lines from (CIMMYT) was conducted in 2014 growing season. The 21 F1 hybrids and one standard check variety (single cross Giza 10) were evaluated in the next season 2015 under two levels of irrigation i.e., normal and water stress conditions. General and specific combining ability effects were estimated according to (Griffing , 1956) , model 1 method 4. Genetic diversity in a tested maize genotypes at molecular level were also assessed using five Inter-Simple Sequence Repeat (ISSR) primers. The six traits studied were ear length, ear diameter, number of rows /ear , No. of kernels/ row, 100 kernel weight, and grain yield /plant. Results displayed that seven crosses ; (P1 X P2 , P1 X P6 , P1 X P7 , P2 X P6 , P2 X P7 , P4 X P5 , P6 X P7) exhibited the most desirable mean values , revealed significant and highly significant positively values of standard heterosis for all traits studied compared with the check variety (Giza 10) beside the significant and highly significant positively data of general and specific combining ability effects under all conditions , in addition to superiority and endurance it has achieved using tolerance indices. The parental line P2 is the best general combiner showing significant desirable GCA effects for the most studied traits under normal and water stress conditions. Significant and highly significant positively of correlation coefficients were showed between ear length and the rest of traits studied under all conditions except the correlation between ear length and ear diameter under water stress conditions where it was negatively correlated , respectively. The genetic diversity using inter simple sequence repeats (ISSR) technique through using five primers for the seven lines of maize was employed giving a total of 23 fragments , where 14 of them were polymorphic bands with 60.86 % polymorphism and 9 fragments was monomorphic bands , respectively.

**Keywords:-** Maize (*Zea mays* L) , , Diallel cross, ISSR markers, Drought tolerance indices , Water stress .

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## INTRODUCTION

Maize is considering one of the most important cereal crop not only in the Middle East and Egypt, but also in all the world and. The importance of this crop in the provision of food, feed and thermal energy of calories for humans, animals and birds, but lately we have noticed a significant decline in the areas of this crop so largely due the effect of drought and dry soil factor. Drought stress is an abiotic factor affecting growth and yields of crop plants and one of the most important limiting factors for maize growth and productivity. The most sensitive period of drought stress in maize located in the period before and after flowering process by about two weeks. This phase is to be that of determining the final number of grains , and exposure to drought during this period directly affect the final number of grains.

[1] Studied dilution drought effects on maize by polyamine and showed that the lowest tolerance stage of growth under drought conditions was the vegetative growth stage at 35 days after planting compounds.

Drought effect will be high in the first growth stage of maize , so genetically engineered technology will improve qualities of maize for drought stress and will open new horizons to face this imminent environmental threat in the crop and other quality attributes. [2] revealed that lowering yield risk is a paramount exporter of advantage of transgenic technology, particularly for accumulated traits. These capitalize are assessment to be tantamount to a yield augmentation of 0.8–4.2 bushels per acre. We observed testimony for gene interactions (yield drag and event lag effects) that can reduce yield. We seek fanciers plant for decades to make every effort to work fruitful for reducing losses in yield due to water stress and all efforts were aimed to improve the varieties of local breeds of maize by breeding programs, such as hybridization and simple selection , using of radiation to get new genes for improving drought tolerance in maize but these attempts took a long time so it was a suitable alternative through the use of genetic engineering and biotechnology for the transfer of recipe resistance of drought and other attributes associated with it , such as yield and its components and succeeded great success in getting maize lines resistant to salinity and water stress , [3-5].

## MATERIALS AND METHODS

### Plant materials

The plant materials used in this study included seven white maize inbred lines introduced from International Maize and Wheat Improvement Center (CIMMYT).These lines i.e. P1, P2, P3, P4, P5, P6 and P7 were sown and crossed in all possible cross combination without reciprocals by hand pollination to obtain grains of 21 F1 straight crosses in 2014 growing season. In 2015 growing season 21 F1 crosses with the check variety (Single cross Giza 10) were evaluated at 1st of June under two different irrigation conditions in two separate field experiments under Egyptian conditions in [Delta region (clay soil) at Al-khanater Experimental Farm EL-Kalyubia Governorate, Egypt.

The first experiment (normal conditions) plants were irrigated every 10 days through the whole season, while the second experiment (drought conditions) was irrigated every 20 days. The two irrigation regimes were applied for each level after 35 days from sowing (i.e. after plants received the first two irrigation).This study was included the project No. (1020105) funded by the National Research Centre , Egypt.

All field experiments lay out in a randomized complete block design with three replications. Wide borders (2m width) have been kept among the different water regimes to minimize the underground water permeability. Each cross was grown in two rows 3 meter long. The spacing between and within rows were maintained at 70 and 20 cm, respectively. All the normal agronomic practices were followed as usual in the ordinary maize field in the areas of study. Data recorded on an individual plant basis for the following traits ; ear diameter (cm) , number of rows /ear , No. of kernels/ row, 100-kernels weight (g) and grain yield/plant (g), respectively.

Analysis of variances was computed to compare the genotypes for each trait in all experiment according to [6], using Costat software package. [7] model 1 method 4 was used to estimate general and specific combining ability effects (GCA&SCA),this method estimates of GCA and SCA from the F1hybrid progenies only. Standard heterosis in addition to drought tolerance and susceptibility indices besides simple

phenotypic correlation (*r*) coefficients among all traits for the entries means were calculated according to [8-16].

\*Abbreviations:- STI : stress tolerance index, YI : yield index, YSI : yield stability index, MP : mean productivity, GMP : geometrical mean productivity, Yr : yield reduction ratio, DSI : drought susceptibility index. Ys : grain yield under drought condition, Yp : grain yield under normal condition.

### Molecular Markers

#### DNA isolation procedure

The bulked DNA extraction was performed using DNeasy Plant Mini Kit (QIAGEN) for the seven lines of maize. Isolation protocol of DNA was as follows:

- 1- Plant tissue was ground using liquid nitrogen to a fine powder, then, the powder was transferred to an appropriately sized tube.
- 2- Then, 400  $\mu$ l of buffer AP1 and 4  $\mu$ l of RNase a stock solution (100 mg/ml) were added to a maximum of 100 mg of ground plant then vortexed vigorously.
- 3- Mixture was incubated for 10 min at 65°C and mixed 2-3 times during incubation by inverting tube.
- 4- Then, 130  $\mu$ l of buffer AP2 was added to the lysate, mixed and incubated for 5 min on ice.
- 5- Lysate was applied to the QIA shredder spin column sitting in a 2 ml collection tube and centrifuged for 2 min at maximum speed (10.000 rpm).
- 6- Supernatant from step 5 was transferred to a new tube without disturbing the cell-debris pellet. Typically, 450  $\mu$ l of lysate was recovered.
- 7- Then, 0.5 volume of buffer AP3 and 1 volume of ethanol (96-100%) were added to the cleared lysate and mixed by pipetting.
- 8- Then, 650  $\mu$ l of the mixture from step 7 was applied through DNeasy Mini spin column setting in a 2 ml collection tube. Then, centrifuged for 1 min at 8000 rpm and flow-through was then discarded.
- 9- DNeasy column was then placed in a new 2 ml collection tube. Then, 500  $\mu$ l buffer AW was added onto the DNeasy column and centrifuged for 1 min at 8000 rpm.
- 10- Then, 500  $\mu$ l buffer AW was added to DNeasy column and centrifuged for 2 min at maximum speed (10.000 rpm) to dry the column membrane.
- 11- DNeasy column was then transferred to a 1.5 ml microfuge tube and 100  $\mu$ l of preheated (65°C) buffer AE was pipetted directly onto the DNeasy column membrane. Then, incubated for 5 min at room temperature and centrifuged for 1 min at 8000 rpm to elute.
- 12- Elution was repeated once as described. A new microfuge can be used for first elute. Alternatively, the microfuge tube can be reused for the second elution step to combine the elutes.

#### Polymerase chain reaction (PCR) condition stock solutions

##### 5X Tris-borate (TBE), pH 8.0

Tris-base	5.40 g
Boric acid	2.75 g
500 mM EDTA, 8.0	0.29 g
H <sub>2</sub> O (d.w) up to	100.00 ml

##### Ethidium bromide

- 1- The stock solution was prepared by dissolving 1 g of ethidium bromide in 100 ml distilled water and mixed well with magnetic stirrer.
- 2- Transferred to a dark bottle and stored at room temperature.

##### Sample loading dye (5x)

Na-EDTA, pH 8.0	(500 mM)	2.00 ml
Glycerol (100%)		5.00 ml

Bromophenol blue (2%)	0.75 ml
H <sub>2</sub> O (d.w.)	1.50 ml

PCR was performed in 30- $\mu$ l volume tubes according to [17] that contained the following:

dNTPs (2.5 mM)	3.00 $\mu$ l
MgCl <sub>2</sub> (25 mM)	3.00 $\mu$ l
Buffer (10 x)	3.00 $\mu$ l
Primer (10 pmol)	2.00 $\mu$ l
Taq DNA polymerase (5U/ $\mu$ l)	0.20 $\mu$ l
Template DNA (25 ng)	2.00 $\mu$ l
H <sub>2</sub> O (d.w.)	16.80 $\mu$ l

**Polymerase chain reaction (PCR) condition for ISSR**

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94° C for 4 min followed by 45 cycles of 1 min at 94° C, 1 min at 57° C, and 2 min at 72° C. the reaction was finally stored at 72° C for 10 min.

**Gel preparation procedure**

- 1- Agarose (1.50 gm) was mixed with (100ml) 1 x TBE buffer and boiled in microwave.
- 2- Ethidium bromide (5 $\mu$ l) was added to the melted gel after the temperature became 55°C.
- 3- The melted gel were poured in the tray of mini-gel apparatus and comb was inserted immediately, then comb was removed when the gel become hardened.
- 4- The gel was covered by the electrophoretic buffer (1 x TBE).
- 5- DNA amplified product (15  $\mu$ l) was loaded in each well.
- 6- DNA ladder (100bp) mix was used as standard DNA with molecular weights of 1500,1200,1000,900, 800, 700, 600, 500, 450, 400, 350, 300, 250, 200, 150 and 100 bp. The run was performed for about 30 min at 80 V in mini submarine gel BioRad .

**Data analysis**

The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among genotypes as revealed by dendrograms were done using SPSS windows (Version 10) program. [18] computer package was used to calculate the pairwise difference matrix and plot the phenogram among cultivars [19].

**Table (1):- List of the primer names and their nucleotide sequences used in the study for ISSR procedure.**

No	Name	Sequence
1	44B	5` CTC TCT CTC TCT CTC TGC 3`
2	98B	5` CAC ACA CAC ACA GT 3`
3	49A	5` CAC ACA CAC ACA AG 3`
4	HB-10	5` GAG AGA GAG AGA CC 3`
5	HB-14	5` CTC CTC CTC GC 3`

**RESULTS AND DISCUSSION**

**Mean Performance:**

The data obtained from half diallel analysis in Table (2), showed that the crosses ; (P1 X P3 , P1 X P4 , P1 X P5 , P1 X P7 , P2 X P7) for ear length and the crosses ; (P1 X P2 , P1 X P5 , P1 X P7 , P2 X P4 , P2 X P5 , P2 X P6 , P2 X P7 , P3 X P4 , P3 X P6 , P4 X P5 , P4 X P6) for ear diameter were the most desirable genotypes for water stress tolerance and achieved high values under normal and water stress conditions , respectively.

**Table (2):- The Mean Performance of maize F1 diallel crosses for all traits studied under Normal and Water Stress Conditions.**

Crosses	Ear Length (cm)		Ear Diameter(cm)		No. of Rows/Ear		No. of Kernels/ Row		100-Kernels weight(gm)		Grain yield/plant (gm)	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
P1 x P 2	17.1	16.74	3.87	3.8	14.67	14.17	31.8	28.67	37.05	28.57	151.38	98.69
P1 x P3	18.7	16.4	3.8	3.59	14.67	12.97	28.67	28.67	27.81	20.21	85.66	73.42
P1 x P 4	18.23	17.93	3.5	3.42	14.17	11.33	25.67	25.67	32.97	30.22	95.59	71.40
P1 x P 5	18.81	11.59	4.7	4.04	14.67	12.67	27.67	19.00	30.97	24.46	90.24	60.88
P1 x P 6	15.94	14.83	4.02	3.87	13.42	11.33	29.53	29.00	33.65	28.26	117.92	71.32
P1 Xp7	19.33	17.41	4.07	3.82	14.06	14	31	27.00	36.15	30.21	142.82	99.92
P2 x P 3	18	15.23	3.63	3.23	13.67	12.67	29.33	29.33	30.07	30.01	85.87	79.85
P2 Xp4	18	16.5	4.45	4.03	14	14	30.33	30.33	30.9	27.35	85.14	83.45
P2 x P 5	17	16.83	4.12	4.11	14.11	12.67	30	30.00	30.87	30.34	98.55	98.36
P2 x P 6	17.33	16.5	4.08	4.07	12.67	12.43	39	32.67	33.52	29.67	142.50	107.49
P2 x P 7	21	17.54	4.5	3.99	13.22	12.67	40	38.00	31.01	30.75	145.08	107.94
P3 x P 4	18	15.97	4.3	3.72	16.13	12	34.67	34.67	30.13	27.47	95.55	83.21
P3 x P5	18.11	15.25	3.8	3.58	14.43	14	31.2	27.33	29.02	28.32	116.91	97.72
P3 x P 6	17.33	13.57	4.54	2.97	14	10.91	30.67	30.67	29.98	22.82	91.99	85.57
P3 x P 7	17.17	14.34	3.74	3.37	14	12.45	31.13	31.00	29.54	21.02	99.19	91.39
P4 x P 5	17.81	17	3.99	3.32	14.01	13.33	33.94	26.67	36.02	28.96	151.00	94.89
P4 x P 6	17.67	15.91	3.87	3.53	12.67	10.97	25.67	25.67	28.94	26.76	71.91	69.50
P4 x P 7	16.5	16.24	3.82	3.19	12.67	12.05	27.99	24.00	30.57	29.63	99.86	85.04
P5 x P 6	16.81	16.67	3.42	3.06	13.33	11.19	26.33	26.33	34.28	29.33	93.69	65.57
P5 x P 7	15.67	15.37	4.02	3.87	12.8	12.67	28.67	21.00	31.64	29.88	96.41	75.54
P6 x P7	16.28	16	4.11	3.17	14.14	11.33	39.67	23.67	30.87	30.69	150.72	74.65
<b>(Giza 1o)</b>	<b>22.5</b>	<b>17.5</b>	<b>3.5</b>	<b>3.92</b>	<b>12</b>	<b>14</b>	<b>40</b>	<b>27</b>	<b>32.72</b>	<b>28.65</b>	<b>137.26</b>	<b>95.39</b>
LSD 0.05	1.63	2.33	0.36	0.53	1.22	1.57	4.52	2.90	1.91	2.42	14.70	9.88
LSD 0.01	2.18	3.12	0.48	0.71	1.64	2.10	6.05	3.89	2.55	3.23	19.67	13.22

On the same side , the crosses ; (P1 X P2 , P1 X P3 , P1 X P4 , P1 X P5 , P1 X P7 , P2 X P4 , P3 X P4 , P3 X P5 , P4 X P5 , P6 X P7) for number of rows/Ear and (P1 X P2 , P2 X P4 , P2 X P6 , P2 X P7 , P3 X P4 , P3 X P7 , P4 X P5 , P6 X P7) for No. of kernels/ row , recorded highest mean values under all conditions , respectively. While the crosses ; (P1 X P2 , P1 X P4 , P1 X P5 , P1 X P6 , P1 X P7 , P2 X P6 , P4 X P5 , P5 X P6 , P6 X P7) revealed the best mean values of 100-grain weight under both treatments of irrigation , respectively, In addition to the crosses , (P1 X P2 , P1 X P7 , P2 X P6 , P2 X P7 , P4 X P5 , P6 X P7) gave the highest mean values of grain yield/plant under the same conditions , respectively. Finally the crosses ; (P1 X P5 , P2 X P5) for ear diameter trait ,(P1 X P2 , P1 X P3 , P1 X P7 , P2 X P4 , P3 X P5) for number of rows/ear , (P2 X P7) for No. of kernels/ row , (P1 X P2 , P1 X P7 , P2 X P6 , P4 X P5 , P5 X P6) for 100-grain weight and the crosses ; (P1 X P2 , P1 X P7 , P2 X P6 , P2 X P7) for grain yield /plant were higher than the check variety (Giza 10) under normal and water stress conditions , while the crosses ; (P1 X P4 , P2 X P7) were higher than the check variety (Giza 10) under water stress only for ear length , respectively .If we examine the results carefully, we find that the following genotypes , (P1 X P2 , P1 X P7 , P2 X P6 , P4 X P5 , P6 X P7) were more powerful and better about the resistance for water stress conditions in maize crop when we compared these results with the control after calculated all ear traits and we found that also these hybrids may represent an important reflection of the strength for the parents involved in their production and hybridized and this of course proves the importance of the additive gene action , which was clearly evident in this crosses superior, So continuation of agriculture these crosses constitute the nucleus to produce maize lines resistant for water stress after revealed the genetic stability and persistence gene. These results were agreement and similar partially with those reported by [20] who studied the basis of variety variances in wheat yield under drought stress and showed that water stress was formed in this rain-free environment by constantly concluding irrigation at several stages before anthesis, beside [21, 22] , [1] where they studied drought stress effect in maize production under different conditions in addition to [23].

### Variation and Interaction:-

Mean squares of half diallel analysis for all traits studied in all maize crosses are revealed in Table (3). The results showed that mean squares of all maize crosses were highly significant for all traits under normal irrigation and water stress conditions and noted that general and specific combining ability effects were highly significant for all traits under all conditions, which confirms that the importance of both additive and non-additive genetic variances. The GCA/SCA ratio was less than the unity for all traits under normal and drought conditions. This revealed that non-additive type of gene action was more an influential and vital in the inheritance and control of these traits for water stress resistance. Therefore, the selection will be prolific using bulk method not pedigree method and It will help to clarify the value of breeding for the selection of the best crosses resistance for water deficit depending on the types of additive gene action through the generations isolationism reaching to the genetic stability [24] and [23].

### Standard Heterosis

Estimates of standard heterosis for 21 maize cross obtained from 7 parents in half diallel analysis by [7], method 4, model 1 for all traits under normal and water deficit conditions was presented in table (4). The cross; (P1 X P4) only under water stress treatment was revealed significant and positive value of standard heterosis for ear length trait, in addition to the crosses; (P1 X P2, P1 X P3, P1 X P6, P1 X P7, P2 X P3, P3 X P4, P3 X P5, P3 X P6, P3 X P7, P4 X P5, P4 X P6, P4 X P7, P5 X P7, P6 X P7) were detected significant and highly significant positively of standard heterosis under normal conditions only for the same trait, while the crosses; (P1 X P5, P2 X P4, P2 X P5, P2 X P6, P2 X P7) revealed significant and highly significant positively of standard heterosis under all conditions of irrigation for ear diameter trait.

On the same side, all crosses exhibited significant and highly significant positively of standard heterosis under normal conditions only for no. of Rows/ear trait, while the crosses; (P1 X P2, P1 X P3, P1 X P6, P2 X P3, P2 X P4, P2 X P5, P2 X P6, P2 X P7, P3 X P4, P3 X P6, P3 X P7) detected the same results under water stress conditions only for number of grains / line trait.

The crosses; (P1 X P2, P1 X P6, P4 X P5, P5 X P6) for the normal irrigation only, (P1 X P4, P2 X P3, P2 X P5, P2 X P7, P4 X P7, P5 X P7, P6 X P7) for water deficit conditions only and the crosses; (P1 X P7, P2 X P6) under the both treatments revealed significant and highly significant positively of standard heterosis for 100-grain weight trait in Table (4), beside the crosses; (P2 X P6, P2 X P7) where achieved the same results under water stress conditions only for grain yield/plant, respectively, which indicated the important of these crosses for SCA effects to oppose water stress and showed the prominence of the three types of gene action (Dominance, Dominance X Dominance, Dominance X Additive) for the inheritance of ear traits in maize, and thus can improve the productivity of maize crop, modify it for drought tolerance and to maintain the food source very high calories, not only for humans but also for birds and animals which beneficial for human. This will only be achieved collect all sources of genetic superiority by crosses, methods of modern genetics and maintain these hybrids until to reach to the genetic stability and inserted into maize breeding programs as parents to transfer recipes higher yielding, resistance for diseases and non-favorable conditions, such as salinity and water stress conditions, [23].



**Table (3):- Mean Squares of Maize F1 diallel Crosses for all Traits Studied under normal and water stress conditions.**

S.O.V	df	Ear Length (cm)		Ear Diameter(cm)		No. of Rows/Ear		No. of Kernels/ Row		100-Kernels weight(gm)		Grain yield/plant (gm)	
		Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Replication	2	4.75	6.81	0.24	0.27	3.14	1.97	1.98	2.35	0.40	0.33	595.41	94.28
Genotypes	20	4.68**	5.97**	0.38**	0.60**	5.22**	3.15**	107.85**	56.55**	29.18**	30.69**	2468.43**	538.26**
GCA	6	3.08**	2.11**	0.18**	0.15**	2.26**	0.84**	45.30**	35.03**	8.80**	15.83**	862.71**	229.80**
SCA	14	0.91**	1.94**	0.11**	0.22**	1.51**	1.14**	31.94**	11.92**	10.12**	7.83**	805.71**	157.83**
Error	40	0.97	2.00	0.05	0.10	0.55	0.90	7.51	3.10	1.33	2.14	79.41	35.85
Error term Mse/r)		0.32	0.67	0.02	0.03	0.18	0.30	2.50	1.03	0.44	0.71	26.47	11.95
GCA/SCA		0.52	0.13	0.20	0.06	0.17	0.07	0.16	0.35	0.10	0.24	0.12	0.17

**Table (4):- Estimates of Standard heterosis of Maize F1 diallel crosses evaluated under normal and water stress Conditions.**

Crosses	Ear Length (cm)		Ear Diameter(cm)		No. of Rows/Ear		No. of Kernels/ Row		100-Kernels weight(gm)		Grain yield/plant (gm)	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
P1 x P 2	-24.00**	-4.34**	10.57**	-3.06**	22.25**	1.21	-20.5**	6.18**	13.23**	-0.27	10.28	3.45
P1 x P3	-16.88**	-6.28**	8.57**	-8.41**	22.25**	-7.35**	-28.32**	6.18**	-15.00**	-29.45**	-37.59**	-23.03**
P1 x P 4	-18.97**	2.45*	0.00	-12.75**	18.08**	-19.07**	-35.82**	-4.92**	0.76	5.47**	-30.35**	-25.14**
P1 x P 5	-16.40**	-33.77**	34.28**	3.06**	22.25**	-9.5**	-30.82**	-29.62**	-5.34**	-14.62**	-34.25**	-36.17**
P1 x P 6	-29.15**	-15.25**	14.85**	-1.27**	11.83**	-19.07**	-26.17**	7.40**	2.84**	-1.36	-14.09	-25.23**
P1 Xp7	-14.08**	-0.51	16.28**	-2.55**	17.16**	0.00	-22.5**	0.00	10.48**	5.44**	4.05	4.74
P2 x P 3	-20.00**	-12.97**	3.71**	-17.60**	13.91**	-9.5**	-26.67**	8.62**	-8.09**	4.74**	-37.43**	-16.29**
P2 Xp4	-20.00**	-5.71**	27.14**	2.80**	16.66**	0.00	-24.17**	12.33**	-5.56**	-4.53**	-37.97**	-12.51*
P2 x P 5	-24.44**	-3.82**	17.71**	4.84**	17.58**	-9.5**	-25.00**	10.00**	-5.65**	5.89**	-28.20**	3.11
P2 x P 6	-22.97**	-5.71**	16.57**	3.82**	5.58**	-11.21**	-2.50	21.00**	2.44*	3.56**	3.81	12.68*
P2 x P 7	-6.66**	0.22	28.57**	1.78**	10.16**	-9.5**	0.00	40.74**	-5.22**	7.32**	5.69	13.15*
P3 x P 4	-20.00**	-8.74**	22.85**	-5.10**	34.41**	-14.28**	-13.32**	28.40**	-7.91**	-4.11**	-30.38**	-12.76*
P3 x P5	-19.51**	-12.85**	8.57**	-8.67**	20.25**	0.00	-22.00**	1.22	-11.30**	-1.15	-14.82*	2.44
P3 x P 6	-22.97**	-22.45**	29.71**	-24.23**	16.66**	-22.07**	-23.32**	13.59**	-8.37**	-20.34**	-32.98**	-10.29*
P3 x P 7	-23.68**	-18.05**	6.85**	-14.03**	16.66**	-11.07**	-22.17**	14.81**	-9.71**	-26.63**	-27.73**	-4.19
P4 x P 5	-20.84**	-2.85*	14.00**	-15.30**	16.75**	-4.78**	-15.15**	-1.22	10.08**	1.08	10.01	-0.52
P4 x P 6	-21.46**	-9.08**	10.57**	-9.94**	5.58**	-21.64**	-35.82**	-4.92**	-11.55**	-6.59**	-47.61**	-27.14**
P4 x P 7	-26.67**	-7.20**	9.14**	-18.62**	5.58**	-13.92**	-30.02**	-11.11**	-6.57**	3.42**	-27.24**	-10.85*
P5 x P 6	-25.28**	-4.74**	-2.28**	-21.93**	11.08**	-20.07**	-34.17**	-2.48	4.76**	2.37	-31.74**	-31.26**
P5 x P 7	-30.35**	-12.17**	14.85**	-1.27**	6.66**	-9.5**	-28.32**	-22.22**	-3.30**	4.29**	-29.76**	-20.80**
P6 x P7	-27.64**	-8.57**	17.42**	-19.13**	17.83**	-19.07**	-0.82	-12.33**	-5.65**	7.12**	9.80	-21.74**
LSD 0.05	1.63	2.33	0.36	0.53	1.22	1.57	4.52	2.90	1.91	2.42	14.70	9.88
LSD 0.01	2.18	3.12	0.48	0.71	1.64	2.10	6.05	3.89	2.55	3.23	19.67	13.22



### General Combining ability effects ((GCA)

The results in Table (5), observed that the best general combiner under normal conditions which exhibited significant and highly significant positive GCA effects were (P1 and P5) for ear length, (P2 and P7) for ear diameter ,(P1) for number of rows /ear , (P2 and P6) for No. of Kernels/ Row, (P1, P2 and P6) for 100 kernel weight and (P1, P2 and P7) for grain yield /plant. On the other hand, under water stress conditions estimates of GCA effects revealed that (P3) for No. of rows/ ear and No. of Kernels/ Row, ( P5 and P7) for 100 kernel weight were the best general combiner for these traits and detected significant and highly significant positive values. The parental line (P2) is the best general combiner showing significant desirable GCA effects for the most studied traits under normal and water stress conditions. Once identified the best parental combiners we can be able to use it in hybridization, as a parent in future maize breeding program to produce the best hybrid combinations under each level of evaluations. The General combining ability effects is the only way to sort out the most important genotypes and parental which can be used as a given the effective and important alleles responsible for resistance to water stress in maize and then can be used in breeding programs, especially in hybridization-breeding operations and on this basis can be considered that parents number; ( P1 , P2 , P7) were the best genotypes for this purpose, [25] and [23].

### Specific combining ability effects

From the data showed in Table (6) and obtained from half diallel analysis, we observed that the crosses ; (P1 X P5 , P5 X P6 , P1 X P7) under drought conditions , (P2 X P7) under all conditions , (P3 X P5) under normal conditions for ear length , (P1 X P6 , P2 X P6 , P3 X P5 , P6 X P7) under normal conditions , (P1 X P5 , P2 X P4 , P2 X P7 , P3 X P6) under water deficit conditions and the cross (P3 X P4) under all conditions for ear diameter , the crosses ; (P1 X P6 , P3 X P4 , P3 X P5 , P6 X P7) under normal irrigation , (P1 X P2 , P1 X P3 , P1 X P6 , P1 X P7 , P3 X P6 , P5 X P6) under water stress for No. of Rows/ear , the crosses ; (P1 X P2 , P2 X P6 , P3 X P5 , P6 X P7) under normal conditions , ( P1 X P6 , P1 X P7 , P2 X P5 , P3 X P4 , P5 X P6) under water stress treatment and (P2 X P7 , P4 X P5) under all conditions for No. of Kernels/ Row , the crosses ; (P1 X P2 , P4 X P5 , P5 X P6) under normal conditions , (P1 X P5 , P2 X P3 , P3 X P4 , P6 X P7) under drought stress and the crosses ; (P1 X P4 , P1 X P7 , P3 X P5) under normal and water stress conditions for 100-grain weight and the crosses ; (P1 X P2 , P1 X P7 , P2 X P7 , P3 X P5 , P4 X P5) under all conditions , (P6 X P7) under normal irrigation and (P2 X P6 , P3 X P4 , P3 X P6 , P5 X P6) under water stress conditions for grain yield / plant detected significant and highly significant positively values of SCA effects and revealed that the importance of (Dominance , Dominance X Dominance and additive X Dominance) types of gene action for the inheritance of these traits and also these hybrids can considered high interest and feasibility in heterosis and traced the genetic across generations isolationism to reach high stress lines for water stress , then introducing these crosses in the Egyptian national program for breeding and production of maize will be important to collect useful genes from all the parents in ways different of hybridization and also the use of modern methods of biotechnology became vital to transfer important traits such as high yield , resistance for salinity, water stress and diseases resistance beside the qualities in this shorthand for the big time in the production of maize lines resistant for stresses , [25] and [23].

### Drought Susceptibility Index (DSI):-

The date in table (7) , revealed that the crosses ; (P1 X P2 , P1 X P4 , P1 X P6 , P2 X P4 , P2 X P5 , P2 X P6 ) for ear length and ear diameter traits , ( P1 X P2 , P1 X P7 , P2 X P6 , P2 X P7 , P3 X P5 , P4 X P7) for number of rows/ear and No. of Kernels/ Row and ( P2 X P3 , P2 X P4 , P2 X P5 , P3 X P4 , P3 X P5 , P4 X P6 , P4 X P7 , P5 X P7) for 100-grain weight and grain yield / plant for example were less than the unity which illustrates and confirms that these hybrids were highly resistance for water stress conditions because the reduction % in grain yield trait was low , while the rest of all genotypes were high susceptible for drought stress because they were higher than the unity and recorded the highest reduction in grain yield trait under stress of drought , respectively.

Table (5):- Estimates of GCA effects for the 7 Parents of Maize evaluated under Normal and Water stress Conditions.

Parents	Ear Length (cm)		Ear Diameter(cm)		No. of Rows/Ear		No. of Kernels/ Row		100-Kernels weight(gm)		Grain yield/plant (gm)	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
P1	0.85**	0.49	0.07	0.09	0.69**	0.11	-0.47	-2.08**	0.99**	-0.36	7.97**	-9.48**
P2	0.11	0.78*	0.13*	0.30**	0.32	0.25	1.41*	4.12**	1.25**	1.29**	11.37**	12.13**
P3	-1.07**	0.02	-0.39**	0.09	0.11	0.65**	-2.30**	2.66**	-2.65**	-3.55**	-18.68**	2.58
P4	0.28	0.21	0.05	-0.17*	0.27	-0.42	-3.93**	-0.28	-0.05	0.56	-11.75**	-4.30**
P5	0.99**	-0.87*	0.09	-0.03	0.24	0.11	-1.48*	-3.61**	-0.52	1.86**	-5.06*	-0.35
P6	-0.79**	-0.90**	-0.10	-0.11	-1.39**	-0.55*	1.60*	-0.08	0.82**	-0.54	-1.92	-2.88*
P7	-0.36	0.26	0.15**	-0.17*	-0.25	-0.15	5.17	-0.74	0.17	0.74*	18.06**	2.29
<b>LSD 0.05</b>	<b>0.47</b>	<b>0.68</b>	<b>0.11</b>	<b>0.15</b>	<b>0.36</b>	<b>0.45</b>	<b>1.31</b>	<b>0.84</b>	<b>0.55</b>	<b>0.70</b>	<b>4.27</b>	<b>2.87</b>
<b>LSD 0.01</b>	<b>0.63</b>	<b>0.90</b>	<b>0.14</b>	<b>0.20</b>	<b>0.47</b>	<b>0.61</b>	<b>1.75</b>	<b>1.12</b>	<b>0.74</b>	<b>0.93</b>	<b>5.69</b>	<b>3.82</b>

If we touched in the depth of the data, we find that these hybrids have proved highly resistant for water stress and may be a result of the compilation of genetic alleles from both parents, this appears clear from the additive and additive X additive types of gene action and the result is that benefit for plant breeders and helps him to select highly tolerance plants of maize for water deficit conditions, [23].

**Tolerance indices:-**

The results obtained in table (8) , revealed that the crosses ; (P1 X P2 , P1 X P3 , P1 X P7 , P2 X P3 , P2 X P4 , P2 X P5 , P3 X P4 , P3 X P5 , P3 X P6 , P3 X P7 , P4 X P5 , P5 X P6) were highly resistance of water stress conditions through the data of (GM , GMP , DTI , YR , YSI , YI) where these genotypes recorded the highest values , while , the crosses ; (P1 X P5 , P4 X P6 , P5 X P6) for (DTI) , (P1 X P3 , P1 X P4 , P1 X P5 , P2 X P3 , P2 X P4 , P2 X P5 , P3 X P6 , P3 X P7 , P4 X P6) for (YR) and the crosses ; (P2 X P3 , P2 X P4 , P2 X P5 , P3 X P6 , P3 X P7 , P4 X P6) for (DSI) recorded the lowest values and were highly tolerance for drought stress because the reduction of yield was low in these genotypes under water stress conditions compared with the control of irrigation treatment unlike other hybrids , respectively, [26 , 27] and [23].

Table (6):- Estimates of SCA effects for the 21F1 Maize diallel Crosses evaluated under Normal and Water stress Conditions.

Crosses	Ear Length (cm)		Ear Diameter(cm)		No. of Rows/Ear		No. of Kernels/ Row		100-Kernels weight(gm)		Grain yield/plant (gm)	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
P1 X P2	-0.72*	-1.56**	-0.16*	-0.37**	-0.18	1.29**	2.09*	-1.44*	3.61**	-0.73	24.75**	8.87**
P1 X P3	0.13	0.80	0.14	-0.23*	-1.17**	0.89*	0.79	0.02	-1.72**	-4.25**	-10.93**	-6.86**
P1 X P4	0.31	0.14	-0.46**	-0.27*	-0.13	-1.38**	-0.88	-0.04	0.85*	1.64**	-7.92*	-1.99
P1 X P5	0.47	1.58**	0.12	0.79**	0.40	-0.58	0.85	-3.38**	-7.19**	1.09*	-19.96**	-16.46**
P1 X P6	-0.61	-2.14**	0.28**	0.03	0.78**	-1.24**	-0.38	3.09**	0.65	0.79	4.58	-3.49
P1 X P7	0.42	1.19*	0.08	0.05	0.29	1.02**	-2.47*	1.76**	3.80**	1.46**	9.49**	19.94**
P2 X P3	-0.30	-0.20	-0.28**	-0.61**	-0.10	-1.24**	-5.55**	-5.51**	0.28	3.89**	-20.14**	-16.01**
P2 X P4	-0.38	-0.39	0.09	0.47**	0.08	1.16**	-4.72*	-1.58*	-1.49**	-2.87**	-23.46**	-9.87**
P2 X P5	-0.59	-0.47	0.14	-0.02	0.21	-0.71*	-3.69**	1.42*	-1.05*	-1.19*	-15.25**	-0.40
P2 X P6	0.69	0.06	0.28**	0.02	0.16	-0.04	7.22**	0.56	0.26	0.55	25.75**	11.07**
P2 X P7	1.30**	2.56**	-0.06	0.52**	-0.18	-0.44	4.66**	6.56**	-1.61**	0.35	8.35*	6.35**
P3 X P4	0.27	0.38	0.28**	0.52**	2.42**	-1.24**	-1.55	4.22**	-1.01*	4.74**	6.34	10.09**
P3 X P5	1.70**	-1.29*	0.33**	-0.34**	0.75**	0.22	6.21**	0.22	1.00*	1.63**	33.35**	8.32**
P3 X P6	-1.06**	0.82	-0.31**	0.70**	-1.15**	0.89*	0.59	0.02	0.62	-1.47**	-1.13	5.11*
P3 X P7	-0.73*	-0.51	-0.17*	-0.04	-0.75**	0.49	-0.50	1.02	0.82	-4.55**	-7.49*	-0.65
P4 X P5	0.05	0.27	0.09	-0.34**	0.16	0.62	10.58**	2.49**	5.41**	-1.84**	60.51**	12.37**
P4 X P6	-0.07	0.97	0.15	-0.05	-1.25**	0.62	-1.41	-2.04**	-3.01**	-1.63**	-21.72**	-10.49**
P4 X P7	-0.17	-1.37*	-0.15	-0.33**	-1.29**	0.22	-2.02*	-3.04**	-0.74	-0.04	-13.74**	-0.12
P5 X P6	0.12	1.05*	-0.69**	-0.30*	-1.00**	0.76*	-10.15**	1.96**	2.79**	-0.37	-34.75**	9.74**
P5 X P7	-1.75**	-1.12*	0.01	0.21	-0.52	-0.31	-3.79**	-2.71**	-0.96*	0.67	-23.89**	-13.57**
P6 X P7	0.94	-0.75	0.29**	-0.41**	2.45**	-0.98**	4.13**	-3.58**	-1.31**	2.12**	27.28**	-11.94**
LSD 0.05	0.72	1.03	0.16	0.23	0.54	0.69	2.01	1.29	0.84	1.07	6.52	4.38
LSD 0.01	0.96	1.38	0.21	0.31	0.72	0.93	2.67	1.72	1.13	1.43	8.68	5.84

**Table (7):- Drought Susceptibility Index (DSI) for All Traits Studied of Maize F1 diallel crosses.**

Crosses	Ear Length (cm)	Ear Diameter(cm)	No. of Rows/Ear	No. of Kernels/ Row	100-Kernels weight(gm)	Grain yield/plant (gm)
P1 X P2	0.21	0.18	0.33	0.57	1.88	1.51
P1 X P3	1.23	0.54	1.14	0.38	2.25	0.62
P1 X P4	0.17	0.22	1.97	0.49	0.69	1.10
P1 X P5	3.85	1.38	1.34	1.82	1.73	1.41
P1 X P6	0.70	0.37	1.53	0.10	1.32	1.71
P1 X P7	1.00	0.60	0.04	0.75	1.35	1.30
P2 X P3	1.54	1.08	0.72	1.39	0.02	0.30
P2 X P4	0.84	0.93	0.14	1.69	0.94	0.09
P2 X P5	0.10	0.02	1.00	0.97	0.14	0.01
P2 X P6	0.48	0.02	0.19	0.94	0.94	1.07
P2 X P7	1.65	1.11	0.41	0.29	0.07	1.11
P3 X P4	1.13	1.32	2.51	2.29	0.73	0.56
P3 X P5	1.58	0.57	0.29	0.72	0.20	0.71
P3 X P6	2.18	3.39	2.17	0.38	1.96	0.30
P3 X P7	1.65	0.97	1.09	0.02	2.37	0.34
P4 X P5	0.46	1.65	0.48	1.25	1.61	1.61
P4 X P6	1.00	0.86	1.32	0.14	0.62	0.15
P4 X P7	0.16	1.62	0.48	0.83	0.25	0.64
P5 X P6	0.08	1.03	1.58	1.68	1.19	1.30
P5 X P7	0.19	0.37	0.10	1.56	0.46	0.94
P6 X P7	0.17	2.24	1.95	2.34	0.05	2.19



Table (8):- Tolerance indices of 21 maize F1diallel crosses.

Genotypes	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI
P1 X P2	151.38	98.69	0.65	1.16	125.03	1.35	122.22	0.35	1.51
P1 X P3	85.66	73.42	0.85	0.86	79.54	0.56	79.31	0.15	0.62
P1 X P4	95.59	71.40	0.74	0.84	63.49	0.62	82.61	0.26	1.10
P1 X P5	90.24	60.88	0.67	0.72	75.56	0.49	74.12	0.33	1.41
P1 X P6	117.92	71.32	0.60	0.84	94.62	0.76	91.70	0.4	1.71
P1 X P7	142.82	99.92	0.69	1.18	121.37	1.29	119.45	0.31	1.30
P2 X P3	85.87	79.85	0.93	0.94	82.86	0.62	82.80	0.07	0.30
P2 X P4	85.14	83.45	0.98	0.98	64.29	0.64	84.29	0.02	0.09
P2 X P5	98.55	98.36	0.99	1.16	98.45	0.87	98.45	0.01	0.01
P2 X P6	142.50	107.49	0.75	0.75	124.99	1.38	123.76	0.25	1.07
P2 X P7	145.08	107.94	0.74	1.27	126.51	1.41	125.13	0.26	1.11
P3 X P4	95.55	83.21	0.87	0.98	89.38	0.72	89.16	0.13	0.56
P3 X P5	116.91	97.72	0.83	1.15	107.31	1.03	106.88	0.17	0.71
P3 X P6	91.99	85.57	0.93	1.01	88.78	0.71	88.72	0.07	0.30
P3 X P7	99.19	91.39	0.92	1.08	95.29	0.82	95.21	0.08	0.34
P4 X P5	151.00	94.89	0.63	1.12	122.94	1.29	119.70	0.37	1.61
P4 X P6	71.91	69.50	0.96	0.82	70.70	0.45	70.69	0.04	0.15
P4 X P7	99.86	85.04	0.85	1.00	92.45	0.76	92.15	0.15	0.64
P5 X P6	93.69	65.57	0.69	0.77	79.63	0.55	78.37	0.31	1.30
P5 X P7	96.41	75.54	0.78	0.89	85.97	0.65	85.33	0.22	0.94
P6 X P7	150.72	74.65	0.49	0.88	112.68	1.01	106.07	0.51	2.19

**Correlation between all Tolerance Indices:-**

If we review the results in a Table (9) and related to tolerance indices through studying grain yield per plant trait under normal and water stress conditions, we find that grain yield under normal irrigation ( $Y_p$ ) was highly significantly correlated with grain yield under water stress conditions ( $Y_s$ ), Correlation analysis between drought indices and yield components revealed that grain yield under irrigated and drought stress condition was positively correlated with (MP, STI, GMP and YI), While, grain yield trait under water stress condition was positively correlated with (YSI) and negatively correlated with (YR) yield reduction ratio and (DSI) drought susceptibility index, respectively. Furthermore, correlation analysis between the various stress tolerant indices used in this study provides interesting observations. (MP, YSI, STI, GMP and YI yield index) detected positively significantly correlated between each other, as well as observed significant negative correlation with (Yr and DSI), respectively. After all this illustration, we can say that tolerance indices is considering one of the strongest pieces of evidence to prove the degree of endurance for water stress or not in the genotypes of maize under the Egyptian conditions by measuring the degree of shortfall and stability in the crop before and after exposure to drought stress and then be the selection process and resumption in agriculture these crosses and repeat the process several times over the generations isolationism to get genetic stability of a viable and very successful. These results are in general agreement with those reported by [28, 29], [26, 27].

**Table (9):- Simple correlation coefficients through tolerance indices between grain yield trait under normal and water stress conditions.**

Indices	GYP	GYS	YSI	YI	GMP	STI	MP	YR	DSI
GYP	1.00								
GYS	0.80**	1.00							
YSI	0.93**	0.77**	1.00						
YI	0.78**	0.83**	0.77**	1.00					
GMP	0.59**	0.72**	0.68**	0.89**	1.00				
STI	0.94**	0.93**	0.80**	0.86**	0.84**	1.00			
MP	0.64**	0.78**	0.33	0.72**	-0.62**	0.90**	1.00		
YR	-0.21	-0.65**	-0.79**	-0.56**	-0.36*	-0.67**	-0.45*	1.00	
DSI	-0.15	-0.23	-0.51**	-0.60**	-0.41*	-0.55**	-0.50**	0.88**	1.00

**Molecular Markers:-**

ISSR data analysis the fragments in the seven promising entries of maize were revealed in presence and absence of fragments on gel photographs figure (1) and table (10) through using five primers detected 23 fragments, where 14 of them were polymorphic bands with 60.86 % polymorphism and 9 fragments were monomorphic bands with 39.13 %.The band size was ranged between 220 to 1350bp. The five primers recorded average of 4.6 bands/ primer.

The first primer (44B) recorded three bands, two of them were polymorphic bands with percentage of 66.66% polymorphism, one band only was monomorphic and range size of bands ranged from 400 to 1350 bp, The fragment with molecular size 400 bp was showed in all entries of maize and molecular size 860 bp was exhibited in the genotypes (2 , 4) , but the entries (5 , 7) were appeared in the molecular size 1350 bp , respectively , which revealed that these molecular sizes were marker for these genotypes of maize.

On the other hand , the second primer (98B) revealed 9 fragments, five of them were polymorphic bands with percentage of 55.55% polymorphism, four bands were monomorphic and range size of fragments was 300 to 1000 bp in addition to the molecular sizes of the fragments (300 , 380 , 430 , 500) bp were observed in all lines of maize , while the molecular sizes (600 , 670) bp were appeared in all genotypes except the entries (P7) and (P1 , P3 , P5) for each molecular size , beside the molecular sizes (760 , 930 , 1000) bp were generated in the line number (4) only which indicated that these fragments were marker for these maize lines for drought tolerance , respectively.

On the same direction the results were showed that the third primer (49A) detected three amplicons, where one of them was polymorphic band with percentage of 33.33 % polymorphism, while the other two fragments were monomorphic and the range size of bands was ranged from 420 to 600 bp, respectively. The first and second fragments were recognized at molecular sizes 420 bp and 580 bp which were appeared in all parents, while the third fragment with molecular size 600 bp was presented in all genotypes except the line number six , which revealed that this fragment was negative marker for this parent of maize.

The results obtained in fig (1) and table (10) revealed that the primer number four and five namely (HB-10 , HB-14) detected four amplicons for each primer , where three of them were polymorphic bands with percentage of 75.0 % polymorphism, while one fragment was monomorphic for the two primers and the range size of bands was ranged from 220 to 930 bp for (HB-10) primer and from 300 to 830bp (HB-14) primer , respectively .

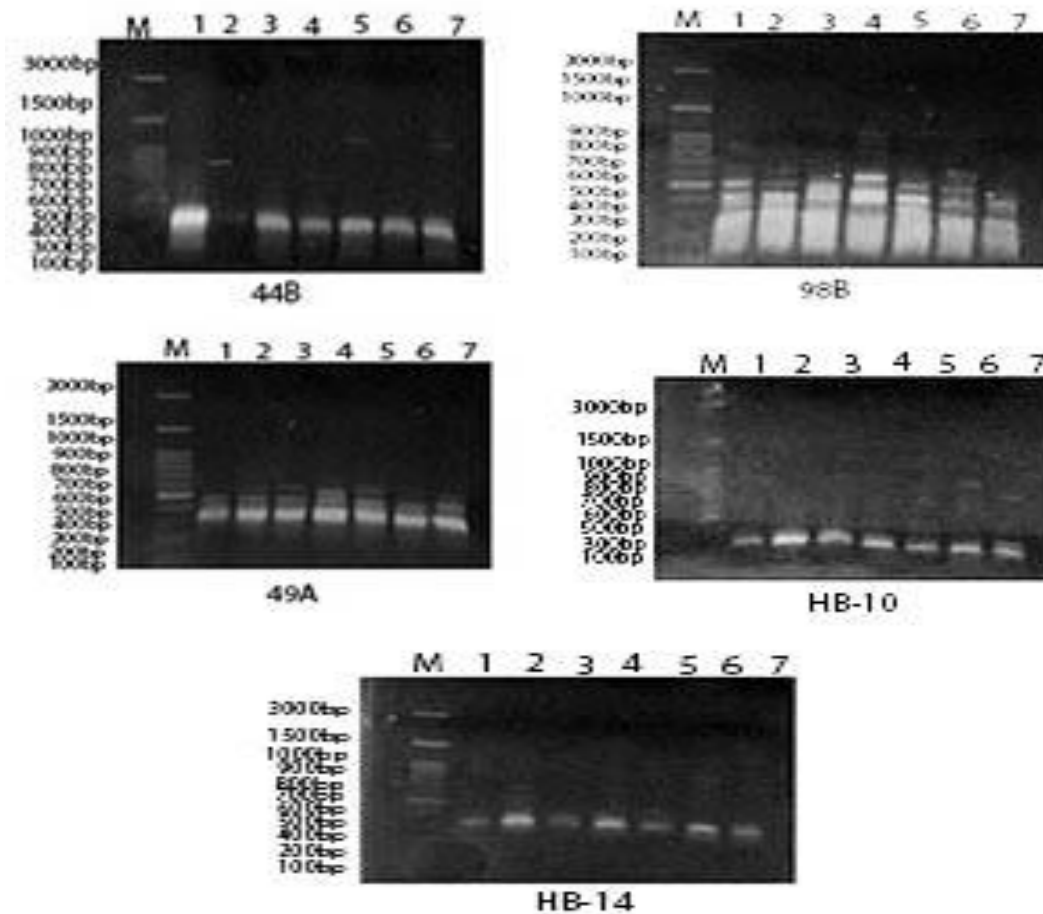
The primer number four (HB-10) characterized all promising lines of maize through four fragments which molecular sizes of them were (220 , 380 , 810 , 930) bp , respectively , where the molecular size of 220 bp was showed in all seven lines of maize , while the rest of molecular sizes for the other three bands were generated in the lines ; (P2 , P3 , P7) , (P7) , (P6) . The bands with molecular sizes of (300 , 360 , 700 , 830) bp were exhibited by primer (HB-14) , where the band with molecular size of 300 bp was showed in all parents , the band with molecular size of 360 bp was generated in the entries (P2 , P3 , P4) , the band with molecular size of 700 bp was presented in the lines ( P2 , P4) , while the band with molecular size of 830 bp was exhibited in the line number (6) only , respectively.

From the previous results can be seen that the primer (49A) recorded the lowest values of polymorphism % , where was revealed (33.33%) , while the primers (HB-10 , HB-14) recorded 75.0% polymorphism for each one of them and considering the highest values of it. On the other hand the primer (98B) recorded nine fragments and considered the highest one for number of bands and exhibited 55.55% polymorphism , while the primers (44B , 49A ) revealed the lowest number of bands (3) for each one of them and revealed (66.66 % , 33.33%) of polymorphism , respectively. Similar investigation were obtained by [30-37] studied RAPD-PCR markers through using two random primers , and revealed 27 amplicons in four species of rice entries ranging from 1600bp to 300bp , while, [38] observed 51 fragments ranging from 2344bp to 160bp by using seven random primers in rice cultivars, On the other contrary [39] exhibited 9 RAPD-PCR reactions using seven genotypes of Egyptian fig and revealed that 111 fragments divided into 39 monomorphic bands and 72 polymorphic bands with 64.86% polymorphism , while [40] showed 71 bands in six lines of wheat using six primers , where 52 of them were monomorphic bands and 19 were polymorphic bands with 26.76% polymorphism , while in this study we revealed 23 fragments , nine of them were monomorphic bands and 14 were polymorphic with 60.86 % polymorphism, respectively. Finally, the combination of all polymorphic bands

(unique or non-unique) were enough to distinguish each of all entries maize under study by one or more unique bands or a group of combined class patterns.

**Table (10):** Total number , Monomorphic, Polymorphic of Bands and Percentage of Polymorphism as Revealed by five ISSR primers on Seven Genotypes of maize.

Primer code	Total bands	Monomorphic bands	Polymorphic bands	Unique bands	polymorphism%	Range size of bands (bp)
44B	3	1	2	0	66.66%	400:1350
98B	9	4	2	3	55.55%	300:1000
49A	3	2	1	0	33.33%	420:600
HB-10	4	1	1	2	75.0%	220:930
HB-14	4	1	2	1	75.0%	300:830
Total bands	23(100%)	9 (39.13%)	8 (34.78%)	6 (26.08%)	60.86 %	328: 942



**Fig. (1):** Banding patterns of Seven maize Entries using Five ISSR primers (44B, 98B, 49A, HB-10 , HB-14); M= 3000 bP:100 bP Ladder Marker.

On the other contrary, the five ISSR Primers exhibited eight markers divided into two negative and six positive markers (Table 11). The negative markers were generated by primers (98B and 49A) at the molecular sizes of (600 and 600) bp for each primer in the parents number (7 ,6) , while the positive markers were detected from the primers ; (98B , HB-10 , HB-14) , where the first primer produced three positive markers at the molecular weights of (1000 , 930 ,760) bp at the parent number (4) , the second primer generated two positive markers at the molecular sizes of (930 , 810) bp in the parents (6 , 7) , while the third primer revealed one positive marker at the molecular size of (830) bp in the parent (6) , respectively , (Table 11). 8 markers among seven maize lines for water deficit stress using five ISSR primers could be applied to genetic linkage



analysis, quantitative trait loci (QTL) mapping, and marker assisted selection (MAS) to development drought resistance in maize and can be exploited in DNA fingerprinting to variety identification.

**Table (11): Negative and positive markers of seven maize genotypes using five ISSR primers.**

ISSR Primer	MS (bp)	P1	P2	P3	P4	P5	P6	P7	MT (N or P)
44B	1350	-	-	-	-	+	-	+	
	860	-	+	-	+	-	-	-	
	400	+	+	+	+	+	+	+	
98B	1000	-	-	-	+	-	-	-	P (P4)
	930	-	-	-	+	-	-	-	P (P4)
	760	-	-	-	+	-	-	-	P (P4)
	670	-	+	-	+	-	+	-	
	600	+	+	+	+	+	+	-	N (P7)
	500	+	+	+	+	+	+	+	
	430	+	+	+	+	+	+	+	
	380	+	+	+	+	+	+	+	
	300	+	+	+	+	+	+	+	
49A	600	+	+	+	+	+	-	+	N (P6)
	580	+	+	+	+	+	+	+	
	420	+	+	+	+	+	+	+	
HB-10	930	-	-	-	-	-	+	-	P (P6)
	810	-	-	-	-	-	-	+	P (P7)
	380	-	+	+	-	-	-	+	
	220	+	+	+	+	+	+	+	
HB-14	830	-	-	-	-	-	+	-	P (P6)
	700	-	+	-	+	-	-	-	
	360	-	+	+	+	-	-	-	
	300	+	+	+	+	+	+	+	
Range	220: 1350 BP	-	-	-	--	-	-	-	-
Total	-	2	7	4	9	3	4	4	2 (N) + 6 (P)

MS: molecular size (bp), MT: marker type, N: negative, P: positive .

Note:- Total is meaning number of positive bands for each parent without monomorphic bands.

**Proximity matrix analysis:**

Estimated the genetic relationships between the seven maize entries were revealed in terms of similarity using Dice coefficient, these results showed within the date presented in Fig.2 and Table (12).

ISSR markers used to figure out the genotypes of maize relationships by UPGMA of the dendrogram and in the Proximity matrix recognized relationships between the promising seven parents.

The similarity ranged from (0.160 to 1.00), where the lowest similarity was (0.160) between (P1 and P3), while the highest values of similarity was (1.00) between (P4 and P7), On the contrary the middle values of similarity were detected among some entries of maize such as (P1 and P2) and (P5 and P6) where the values were (0.520, 0.560), respectively.

The entries number (4, 5, 6, 7) were considering the biggest proof of genetically convergence among these genotypes under study and the first responsibility for producing hybrids high resistance for water stress because these lines were highly performance under stress conditions. , So using of these entries in maize breeding programs for drought tolerance will be fruitfully and more effective.

**Table (12):- Genetic Similarity matrix between seven genotypes of maize with ISSR markers based on Jaccard coefficients.**

Case	P1	P2	P3	P4	P5	P6	P7
P1	1.00						
P2	0.520	1.00					
P3	0.160	0.230	1.00				
P4	0.690	0.280	0.640	1.00			
P5	0.00	0.610	0.290	0.760	1.00		
P6	0.450	0.690	0.660	0.830	0.560	1.00	
P7	0.450	0.690	0.410	1.00	0.290	0.870	1.00

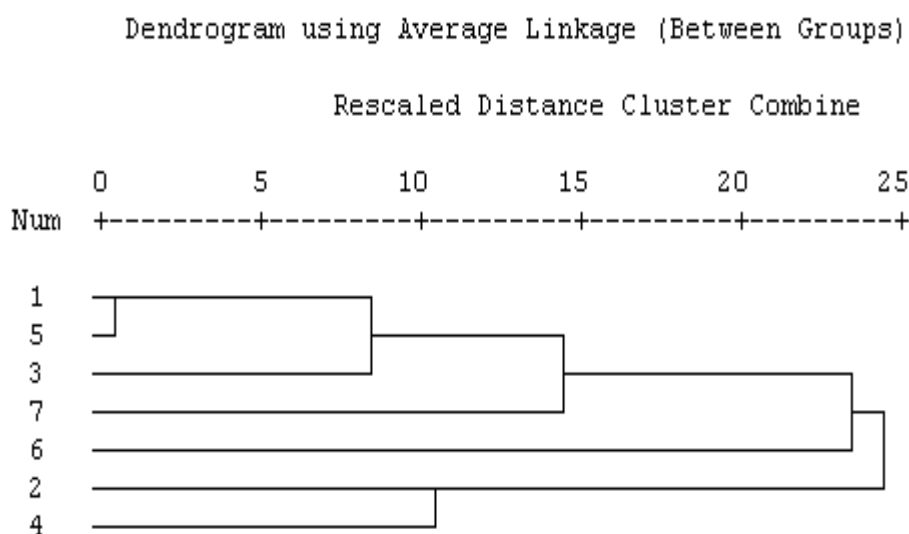
**Genetic Similarity:-**

The present study aimed to know the ISSR Markers competence in revealing and strictly the genetic relationships between the seven maize entries using five primers table (12). Genetic similarity ranged from (0.160 to 1.00) and the mean value of genetic similarity was (0.580) including 21 pairwise comparisons among the seven entries of maize, where the genetic similarity was 100% between (P4 and P7) , while it was 0% among (P1 and P5) based on 23 bands , 9 of them were monomorphic bands and 14 were polymorphic bands with 60.86 % polymorphism , respectively.

The phylogenetic tree of cluster analysis divided into two main groups , the first one divided into one sub-group and involved (P2 , P4) , while the second main group embraced the rest of the seven genotypes of maize , where divided into two sub-groups ., the first one was (P6) , but the second sub-group divided into three classes, the first class included (P1 , P5) , the second class included (P3) , while the third class was (P7) only in fig (2) , respectively.

Similar investigations were in agreement with those revealed by [41 , 42] who studied and observed that any genotypes from the same geographical area were divided in to different clusters.

From the previous results it could be concluded that the phylogenetic tree proved that the maize entries used in this study were highly tolerance for water stress especially (P4 , P5 , P6 , P7) and these results confirmed from the data obtained from all traits studied under drought stress compared with the control like (the mean performance of the crosses obtained from these parents and final analysis of half diallel design .,Standard heterosis , general and specific combining ability effects in addition to tolerance indices of water stress calculated from diallel maize crosses).



**Fig (2):- Dendrogram using average Linkage (Between Groups) Rescaled Distance Cluster Combine of Genetic Relationships among seven Entries of maize.**

## CONCLUSION

21 cross of maize were grown under normal and water stress conditions to estimate some ear traits such as ; ear length , ear diameter , number of rows / ear , number of grains/line , 100-grain weight and grain yield/plant under normal irrigation and water stress conditions and evaluated some genetic parameters through half diallel analysis using (Griffing , , model 1,method 4) in addition to study the genetic diversity between the seven parents of maize using five primers of (ISSR) markers. The results revealed that the seven crosses ; (P1 X P2 , P1 X P6 , P1 X P7 , P2 X P6 , P2 X P7 , P4 X P5 , P6 X P7) detected the most desirable mean values under water deficit conditions compared with the control treatment and ISSR markers characterized the seven parents of maize through giving 23 fragments , where 14 of them was polymorphic bands with 60.86% polymorphism and the rest fragments (9) were monomorphic bands , So the crosses obtained from these entries are considering to be the foundation of the escalation for the genetic stability of access to produce tolerance maize lines for water deficit conditions under Egyptian conditions.

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