

## Determination of Combining Ability and Genetic Diversity Using ISSR Markers to Evaluate the Genetic Variability in Wheat

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### ABSTRACT

#### Key words:

Wheat, ISSR marker, combining ability, drought stress, GCA and SCA.

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Eight bread wheat genotypes were screened by the six ISSR primers and scored a total of 54 amplified DNA bands. The number of bands varied from 40 to 69 bands from primers ISSR 2 (6 fragments) to ISSR 5 (13 fragment). Number of polymorphic fragments varied from 1 for ISSR 4 to 8 for ISSR 1 and ISSR 5. The six ISSR primers generated 314 scorable bands across 8 studied genotypes. These primers produced a total of 54 reproducible fragments, from which 30 (55.55%) were polymorphic. The lowest genetic similarity (0.63) was obtained between P3 and P7, while, the highest genetic similarity (0.89) was scored between P7 and P8. The parents were crossed in a 8x8 half diallel scheme in 2015/2016. Parents and their 28 F<sub>1</sub> crosses were evaluated under normal and stress conditions during 2016/2017 in two irrigation levels experiments. The mean squares were significant for all studied traits. The highest mean values were detected by parents P2, P2, P8, P2, P6 and p2 for plant height, spike length, no of spike/ plant, 1000-kernel weight, biological yield/ plant and grain yield/ plant in the combined analysis, respectively. While, the highest mean values were recorded under combined analysis with crosses P1xP6 for biological yield/ plant and the cross P2xP4 for grain yield / plant. Mean squares for combining ability estimates were highly significant for all studied traits. The ratios GCA /SCA exceeded the unity for all studied traits, revealing that additive and additive x additive types of gene action are more important than non-additive gene action in controlling these traits. The parental P6 exhibited positive and significant  $\hat{g}_i$  effects for grain yield/ plant and its attribute. The highest desirable SCA effects were obtained with the crosses P1xP6, P1xP7, P2xP4, P3xP7, P3xP8, P4xP8, P5xP8 and P6xP7 for grain yield/ plant which exhibited significant and positive  $\hat{s}_{ij}$  effects. Positive correlation coefficients were found between genetic diversity and each of mean performance and SCA for grain yield/plant. Hence, ISSR marker can be used as a tool for determining the extent of genetic diversity among wheat genotypes and can be used to precisely predict the yield performance value for F<sub>1</sub> hybrids.

### تحديد القدرة على التآلف و التباعد الوراثي باستخدام تقنية ISSR لتقييم التنوع الوراثي في القمح

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#### الكلمات المفتاحية:

استخدمت ستة بوايد ISSR على الثمانية تراكيب وراثية تحت التجربة. أظهرت النتائج أن البادى

القدرة على التآلف، التباعد الوراثي، ISSR 2 حقق أعلى عدد من الحزم في جميع الآباء تحت الدراسة. بينما أظهر البادى ISSR 5 أقل عدد من الحزم. تراوح العدد الكلى لشظايا الـ DNA (Fragments) بين 6 شظايا للبادى ISSR 2 الى

13 شظاية للبادى ISSR5. تراوح العدد الكلى لشظايا الـ DNA المختلفة ( Polymorphic

للمراسلة:

<sup>1</sup> This paper is a part of MSc. Thesis for the first author

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fragments) من 1 في البادئ 4 ISSR الى 8 في البادئ 1,5 ISSR. كان العدد الكلي لشظايا الـ DNA المختلفة للآباء الثمانية 30 بنسبة 55,55%.

التماثل الوراثي: تراوح التماثل الوراثي ما بين 0.63% لآبوين P3 و P7 الى 0.89 بين الآبوين P7 و P8. أوضح التحليل العنقودي Cluster analysis ان هناك علاقات وراثية للتماثل بين الآباء الثمانية تحت الدراسة حيث تم تقسيم الآباء الى قسمين رئيسيين Two main cluster. أحتوى الاول على الاب رقم 2 اما الثاني احتوى على باقي الآباء. ولدراسة قوة الهجين والقدرة على التألف لصفات المحصول ومكوناته لثمانية آباء من القمح بالإضافة إلي 28 هجين ناتجة منها بنظام Half diallel وذلك في محطة تجارب بحوث كلية زراعة مشتهر جامعة بنها، حيث تم عمل تجربتين بمزرعة الكلية. في التجربة الأولى تم الري مرة واحدة بعد رية الزراعة بينما التجربة الثانية تم إجراء معاملات الري الطبيعية، دونت البيانات على عشرة نباتات فردية أخذت عشوائيا من كل قطعة تجريبية وقدرت قوة الهجين لكافة الصفات المدروسة كنسبة مئوية لإنحراف قيمة الهجين عن قيمة متوسط الأبوين أو قيمة الأب الأفضل. وتم تحليل البيانات باستخدام طريقة الهجن التبادلية (جرفنج 1956) الطريقة الثانية الموديل الأول. وكانت الصفات المدروسة هي: طول النبات (سم) - طول السنبلة - عدد سنابل النبات - وزن 1000 حبة - المحصول البيولوجي - محصول الحبوب/ نبات (جم). كان التباين الراجع للتراكيب الوراثية الآباء والهجن والتفاعل بين الآباء والهجن معنويا لكل الصفات المدروسة تحت ظروف التحليل المشترك.

أظهرت كلا من الآباء P2, P6, P2, P8, P2, P2 أعلى قيم لصفات طول النبات، طول السنبلة، عدد السنابل / النبات، وزن 1000 حبة، المحصول البيولوجي/ نبات ومحصول حبوب النبات الفردي علي التوالي. كما أظهر الهجين P1xP6 أعلى قيم لصفة المحصول البيولوجي للنبات و الهجين P2xP4 لصفة محصول الحبوب للنبات. كان التباين الراجع للقدرة العامة والخاصة على التألف معنويا للصفات تحت الدراسة. كانت النسبة بين القدرة العامة/القدرة الخاصة أعلى من الوحدة للصفات تحت الدراسة في كل من معاملتي الري والتحليل المشترك. وأظهرت السلالة P6 قدرة عامة علي التألف لصفات طول السنبلة، عدد السنابل للنبات، ووزن 1000- حبة والمحصول البيولوجي للنبات و محصول حبوب النبات. أظهرت الهجن P1xP6, P1xP7, P2xP4, P3xP7, P3xP8, P4xP8, P5xP8, P6xP7 بالنسبة لصفة محصول النبات الفردي قدرة خاصة علي التألف معنوية.

أظهر تحليل الارتباط أن هناك معنوية بين التباعد الوراثي وكل من متوسط أداء الهجن وقوة الهجين بنوعها أي لمتوسط الآبوين والاب الأفضل وتأثير القدرة الخاصة على التألف لكل الهجن تحت الدراسة موجبة ولكن منخفضة.

من خلال هذه الدراسة يتضح ان تكنيك ISSR من المعلمات الجزيئية يمكن أن يستخدم في تحديد التباعد الوراثي بين الآباء الثمانية وتقسيمها الى مجموعات واستخدام هذا التباعد في التنبؤ بالمحصول وقوة الهجين للهجن الناتجة بين هذه الآباء.

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is the major cereal crop in Egypt and several other countries. World average cultivated area of wheat was 221.73\* <sup>2</sup>million hectares in 2017\*; the total production was 751.36\* million metric tons, with an average productivity of 3.39\* metric tons

\*Foreign Agricultural Service / USDA Office of Global Analysis

<http://www.pecad.fas.usda.gov>

-(1 metric ton per hectare = 100 grams per square meter, 1 hectare (ha) = 10,000 square meters).

hectare<sup>-1</sup>. In Egypt wheat grew in 1.25\* million hectares that produced 8.10\* million metric tons of grains, with an average yield of 6.43\* metric tons hectare<sup>-1</sup>. With increasing population, it could hardly satisfy only 55% of local requirements. The increasing gap between production and consumption necessitates increasing wheat production in Egypt. To overcome this problem is to increasing the productivity of wheat through an efficient breeding program.

Stresses can occur at any stage of plant growth and development, thus illustrating the dynamic nature of crop plants and their productivity is needed. Drought among abiotic stresses is the most widespread and limiting crop productivity. There are definitions of drought, which include precipitation, evapo-transpiration, potential evapotranspiration, temperature, humidity and other factors individually or in combination (**Renu and Suresh, 1998**). Also selection for genotypes with increased productivity in drought environments has been an important goal of many plant breeding programs, the biological basis for drought tolerance is still poorly understood.

The diallel cross designs are frequently used in plant breeding research to obtain information about genetic properties of parental lines or estimates of general combining ability (GCA), specific combining ability (SCA) and heritability (**Baker, 1978; EL- Maghraby et al., 2005 and Iqbal et al., 2007**). In addition, the diallel cross technique was reported to provide early information on the genetic behavior of these attributes in the first generation (**Chowdhry et al., 1992 and Topal et al., 2004**). Diallel analysis technique is the choice of providing such detailed genetic information for selecting breeding materials that show great promise for success (**Lonnguit and Gardner, 1961**).

Molecular markers that reveal polymorphism at the DNA level have been shown to be a very powerful tool for genetic diversity since they were independent of the confounding effects of environmental factors. Molecular techniques are now a valuable tool for achieving genetic variation among wheat parents is necessary to derive superior progeny from crossing and selection. Precise information on the nature and degree of genetic diversity present in wheat collections from its principal areas of cultivation would help to select parents for evolving superior varieties.

New molecular tools such as inter simple sequence repeats (ISSR) have now provided the opportunity to monitor genetic integrity at the genotype level and laboratory tests are available to determine any unintentional genetic erosion or change in genetic identity. Therefore, the investigation aimed to: use ISSR-PCR marker to detect DNA polymorphism, identify parents and estimate genetic diversity among wheat genotypes and assess the variations amongst a half diallel crosses among eight genotypes for drought avoidance and drought tolerance traits.

## MATERIALS AND METHODS

**Plant materials:** Eight genotypes of wheat representing a wide range of diversity for several agronomic characters and drought resistance measurements were selected for the study. The names, pedigree and origin of these varieties are presented in Table (1).

**Table (1): The name, pedigree and source of the studied parental varieties and lines.**

NO	Entry name	Pedigree	Source
1	Yakora Rojo	Ciano 67/Sonora 6411 Klien Rendidor/3/1L815626Y-2M-1Y-0M-302M	CIMMYT
2	Gemiza 7	CMH74 A. 630/5x/Seri 82/3/Agent (Gemiza 7)	Egypt
3	Giza 168	MRI/BUG/SEPI CM933046-8M-OY-OM•2Y-O3-OGZ.	Egypt
4	Gemiza 11	BOW"S"/KVZ"S"/7C/SERI82/3/GIZA 168/SAKHA61. GM7892-2GM-1GM-2GM-1GM-0GM.	Egypt
5	Sakha 93	S 92/TR 810328 S8871-1S-2S-1S-0S	Egypt
6	Sides 12	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL /4/CHAT"S"/6/MAYA/VUL/CMH74A.630/ 4*SXSD7096-4SD-1SD-1SD 0SD	Egypt
7	Sahel 1	NS 732/PIMA//Veery'S'	ICARDA
8	13-ssd-43	Landraces	Egypt

Line No 8 was developed in the Department Of Agronomy , Faculty of Agric. at Moshtohor , Banha Univ. by Prof. Dr. M. El.Badawy.

**DNA extraction:** 15 seeds of parental genotypes were sowing in pots. Leaf tissue was collected from 5-7 days old germinated seedlings. Equal quantities of leaf tissue from 10 seedlings of each genotype were bulked, lyophilized and ground with a mortar. Genomic DNA was isolated and extracted using mi-plant genomic DNA Isolation Kit (Metabion).

**Inter-simple sequence repeats (ISSR):** After estimating the DNA samples concentration aliquots from each stock of DNA samples were diluted to a uniform concentration of 10 ng/μl to be used with PCR marker. Oligonucleotide sequences of the primers used in this study were selected from a set of Operon kits (Operon Technologies Inc., Alameda California, USA). A total of six primers as indicated in Table (2) were used in the detection of polymorphism among eight wheat genotypes.

Reactions were carried out in a total volume of 25 μl containing 30 ng of genomic DNA as a template, 30 pmoles of random primer, 2mM of dNTP's mix (dATP, dCTP, dTTP and dGTP, ABgene, Surrey, UK), 10 X PCR buffer, 25 mM MgCl<sub>2</sub>, and 2 units Taq DNA polymerase (promega, USA).

Amplifications were carried out in a thermo cycler (UNO II Biometra) programed for 94°C FOR 4 min, (one cycle); followed by 94°C for 45 sec, 38°C for 1 min, and 72°C for 1 min (35 cycle); 72°C for 10(one cycle) ,then 4°C(infinite).

The amplification products were resolved by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5 μg/ml), visualized with ultraviolet light and photographed. DNA fragment sizes were determined by comparison with the 100bp and 1Kb DNA ladder marker (promega USA).

**Field experiments:** This investigation was carried out at the Experiment, Research Station of Moshtohor Faculty of Agriculture, Benha University, Kalubia Governorate, Egypt. In 2015/2016 growing season, the parents were crossed in a 8x8 diallel cross excluding reciprocals giving a total of twenty-eight crosses. In 2016/2017 two experiments using randomized complete block design with three replications were carried out. Each experiment contained the eight parents and their resulting 28 F1's. The sowing date was on 4<sup>th</sup> Dec. 2016. The first experiment was irrigated only once after planting irrigation and the second one was normally irrigated 5 irrigations. Plots of parents and F1's consisted of one row, 3 m-long, with spacing of 30 cm between rows and 20 cm between plants. The dry method of planting was used in this study. The other cultural practices of growing wheat were practiced. The amounts of total rainfall during the evaluating season were recorded in Table (2).

**Table 2. Monthly averages of temperature, relative humidity (R.H.) and total rain fall during 2016/2017 season at Kalubia (Moshtohor).**

Months	Temperature C		R.H. (%)	Rain fall mm/month
	Min.	Max.		
Dec.2016	19.7	9.2	51.3	0.5
Jan.2017	17.7	6.1	55.9	1.6
Feb.2017	20.4	7.8	47.2	0.8
Mar.2017	25.8	11.4	37.3	0.4
Apr.2017	29.1	14.4	38.9	0.3
May.2017	34.5	19.0	32.1	----

According to meteorological weather station Moshtohor.

Ten guarded plants from parents and the F<sub>1</sub>'s were selected randomly from each plot for recording observations on different characters. The characters studied were, Plant height(cm), No .of spikes /plant, No .of kernels/ spike, 1000- kernel weight (g), biological yield/ plant and grain yield/ plant (g).

**Data analysis:** Analysis of variance was conducted as outlined by **Steel and Torrie (1980)** for all characters. The analysis of GCA and SCA was done following the procedure given by **Griffing (1956)** using Method II Model I. The combined analysis of the two experiments was carried out whenever homogeneity of mean squares was detected (**Gomez and Gomez 1984**).

**ISSR analysis:** The obtained data of ISSR analysis were entered in a computer file as binary matrices where 0 stands for the absence of a band and 1 stands for the presence of a band in each individual sample. Similarity coefficients between a pair of inbred lines were calculated according to Jaccard (1908). A dendrogram tree was constructed by the UPGMA clustering algorithm from the SAHN option of NTSYS-PC version 2.1 (**Rohlf, 2000**).

## RESULTS AND DISCUSSION

**ISSR Polymorphism:** ISSR experiments were conducted using ten specific primers. Four primers gave non-polymorphic fragments. Meanwhile six primers gave polymorphic amplification products; these are primer ISSR1, ISSR2, ISSR4, ISSR5, ISSR6 and ISSR10. Therefore, the last six primers were included in this study. Also, ISSR fingerprint for the studied genotypes were done to study the diversity and relationship between molecular marker polymorphism and mean performance. Among the 54 fragments generated, that ranged from six for ISSR2 to 12 for ISSR1. The mean of fragments per primer was 9. The size of fragments ranged from 20.00 b.p to 340.21 b.p length. Among 54 generated fragment 30 were polymorphic fragment obtained within the eight genotypes (Table 3). Other studies indicating different results **Guo-yue and Hui (2007)** , **Sharma et al.(2014)**, **Tonk et al.(2014)**, **Razmjoo et al. (2015)**, **Tarinejad et al.(2015)**, **Olgun et al.(2015)** and **Chaudhary et al.(2016)**.

**Table (3) primer used in ISSR analysis of eight wheat genotypes and total number of fragments detected by each primer and number of polymorphic fragments.**

Primers ISSR	Sequence	TSB	TF	NPF	PPF
1	5'-AGAGAGAGAGAGAGAGYC-3'	65	12	8	66.67
2	5'-AGAGAGAGAGAGAGAGYG-3'	40	6	4	66.67
4	5'-ACACACACACACACACYG-3'	55	7	1	14.29
5	5'-GTGTGTGTGTGTGTGTGYG-3'	69	13	8	61.54
6	5'-CGCGATAGATAGATAGATA-3'	44	8	4	50.00
10	5'-GACAGACAGACAGACAAT-3'	41	8	5	62.50
Total		314	54	30	-
Mean		52.33	9.00	5.00	53.61

Where: TSB = Total number of scorble bands, TF= Total number of fragments, NPF = Number of polymorphic fragments, PPF = Percentage of polymorphic fragments.

**Genetic similarity:** The genetic similarity matrix was produced from the ISSR data using **Nei and Li's formula (1979)**. Genetic similarity coefficients are presented in (Table 4). The lowest genetic similarity (0.63) was obtained between the two parental genotypes P3 and P7, while, the highest genetic similarity (0.89) was scored between the two parental genotypes P7 and P8. The over all mean for genetic similarity among all genotypes was (0.74). **Tahir (2010)** analyzed eleven wheat varieties of diverse origins with 12 selected ISSRs and found a wide range of genomic diversity among all the genotypes, indicating them as ISSR prime candidates for selective breeding for

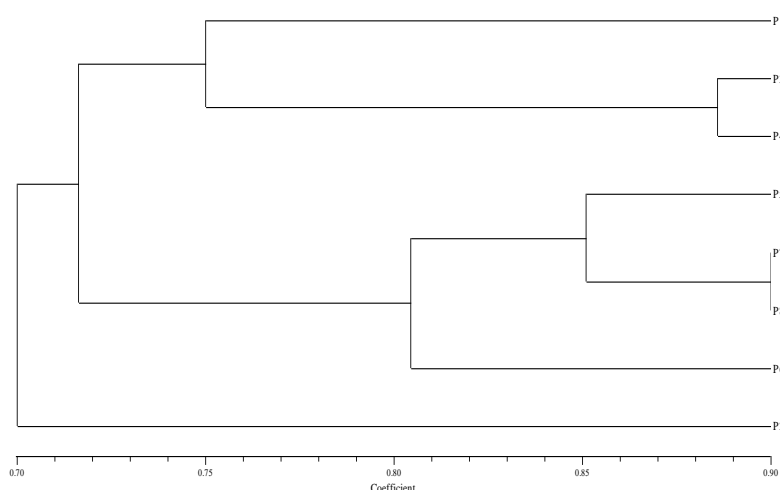


specific traits and broadening the genetic base. Also, Abou-Deif *et al.* (2013) Analyzed 20 wheat genotypes using (ISSR) markers and found ISSR markers succeeded in distinguishing most of the 20 varieties in relation to their genetic background and geographical origin.

**Cluster analysis:** The dendrogram constructed from cluster analysis based on ISSR data is represented in Fig (1). The data collectively distinguished two main clusters. The first main cluster contained Gemiza 7 (P2) while, the second cluster consists of remaining

**Table (4): Genetic similarity based on Nei and Li's coefficient for eight genotypes in wheat revealed by ISSR.**

Rows/ cols	p1	p2	p3	p4	p5	p6	p7	p8
p1	1.000							
p2	0.750	1.000						
p3	0.740	0.640	1.000					
p4	0.765	0.700	0.884	1.000				
p5	0.700	0.702	0.727	0.837	1.000			
p6	0.760	0.694	0.681	0.782	0.833	1.000		
p7	0.647	0.681	0.630	0.733	0.825	0.767	1.000	
p8	0.686	0.761	0.674	0.777	0.875	0.814	0.897	1.000



**Fig. (1): phonogram generated by UPGMA cluster analysis based on Nei and Li' coefficients showing clustering of eight genotypes.**

seven genotypes this cluster separated into two sub-clusters: the first sub-cluster contained three genotypes Yakora (P1), Giza 168 (P3) and Gemiza 11( P4). Meanwhile, the second sub cluster contained the other four genotypes i.e. Sakha 93 (P5), Sahel 1( P7), 13-ssd-43 (P8) and Sides 12(P6) . In addition, the second sub cluster divided into two sub-sub clusters the first sub-sub cluster contained P6. While, the inbred lines P5, P7 and P8 were belonging to the second sub-sub cluster as well as the two genotypes 7 and 8 being closely related.

**The second experiment:** Analyses of variance for yield and its components under drought and normal irrigation and combined analysis across the mention environments are presented in Table 3. Results indicated that mean squares due to irrigation treatments (Environments) were highly significant for all studied traits indicating overall differences between the two environments of study.

Genotypes mean squares were significant for all studied traits indicating wide diversity between all genotypes used in this work. Moreover, significant mean squares between genotypes and environment interaction were detected for No of spikes/plant, biological yield/ plant and grain yield/ plant. This result indicated that genotypes responded differently to different environments for the mention traits.

Mean squares due to parents were highly significant for all traits in drought stress, normal irrigation and combined across them indicating that these parents are differently in the aforementioned significant traits. Moreover, mean squares due to the interaction between parents and environments were significant for No of spike/ plant, Biological yield/ plant and grain yield/ plant. Such result indicated that wheat parents responded differently to stress and non-stress conditions for these traits. For the exceptional traits, insignificant mean squares between parents and environments were detected indicating that parents behaved similarly in stress and non-stress conditions.

### Mean performance

Results in Table (5) showed the average of plant height, yield and its components traits at the combined across irrigation treatments. It's clear that the parental line (P<sub>1</sub>) gave the lowest mean value for plant height. On the other hand, P<sub>2</sub> was the tallest parent. Plant height for crosses ranged from 72.50 cm (P<sub>1</sub>xP<sub>3</sub>) to 97.75cm (P<sub>6</sub>xP<sub>7</sub>). Moreover, the crosses P<sub>2</sub>xP<sub>4</sub>, P<sub>2</sub>xP<sub>7</sub>, P<sub>3</sub>xP<sub>7</sub>, P<sub>4</sub>xP<sub>5</sub>, P<sub>4</sub>xP<sub>8</sub> and P<sub>6</sub>xP<sub>8</sub> did not differ significantly than the tallest hybrid P<sub>6</sub>xP<sub>7</sub>. Some farmers usually prefer higher plant due to the high price of hay.

**Table (5) Mean squares for yield and its components under drought stress condition and normal irrigation as well as the combined over them.**

S.O.V.	df	plant height	spike length	No. of spikes /plant	1000 kernel weight	Biological yield/plant	Grain yield/plant
<b>Drought environment</b>							
Rep	2	41.82	7.34**	17.81*	100.51**	7.96	0.3
Genotypes(G)	35	163.82**	2.55**	102.81**	56.12**	4046.34**	141.80**
Parent (P)	7	123.61**	2.62	95.07**	73.90**	2320.86**	119.96**
Cross ( C)	27	178.29**	2.53*	90.55**	53.37**	4247.56**	152.00**
P vs C.	1	54.57*	2.67*	488.02**	5.76	10691.64**	19.1*
Error	70	28.19	1.3	5.49	16.02	77.59	9.23
<b>Normal environment</b>							
Rep	2	7.16	1.9	3.39	5.33	2.16	6.72
Genotypes(G)	35	176.69**	4.50**	114.25**	70.14**	4683.45**	179.64**
Parent (P)	7	288.20**	4.69**	70.93**	114.22**	7645.45**	182.67**
Cross ( C)	27	146.43**	4.58**	125.90**	60.67**	4012.33**	180.58**
P vs C.	1	213.00**	0.93	102.93**	17.19	2069.68**	132.89**
Error	70	23.56	1.18	4.58	9.18	80.31	14.61
<b>Combined analysis</b>							
Irrigation (I)	1	5726.03**	102.78**	2681.12**	443.19**	363533.47**	3985.17**
Rep/ I	4	24.49	4.62**	10.6	52.92**	5.06	3.51
Genotypes(G)	35	301.97**	5.70**	155.57**	117.43**	6221.16**	230.37**
Parent (P)	7	374.62**	6.63**	154.87**	181.57**	7009.63**	254.07**
Cross ( C)	27	285.38**	5.55**	142.26**	104.35**	5836.61**	228.07**
P vs C.	1	241.60**	3.37*	519.60**	21.42	11084.74**	126.37**
G x I	35	38.54	1.34	61.50**	8.83	2508.63**	91.07**
p x I	7	37.2	0.68	11.12*	6.55	2956.68**	48.57**
C x I	27	39.35	1.55	74.19**	9.69	2423.28**	104.51**
P.vs.C x I	1	25.97	0.22	71.35**	1.52	1676.59**	25.62
Error	140	25.87	1.24	5.04	12.6	78.95	11.92

\* and \*\* significant at 0.05 and 0.01 levels of probability, respectively.

On the other hand, this plant must be given high yield for grain and behave resistant to lodging. The highest parents mean value for spike length (12.92cm) was detected for P<sub>2</sub>. However, eight crosses P<sub>2</sub>xP<sub>3</sub>, P<sub>2</sub>xP<sub>4</sub>, P<sub>3</sub>xP<sub>4</sub>, P<sub>3</sub>xP<sub>7</sub>, P<sub>4</sub>xP<sub>6</sub>, P<sub>4</sub>xP<sub>7</sub>, P<sub>4</sub>xP<sub>8</sub> and P<sub>7</sub>xP<sub>8</sub> exhibited highest values for spike length. For No. of spike/ plant the parent P<sub>8</sub> and the cross P<sub>1</sub>xP<sub>4</sub> give the highest number of spikes/ plant. Heavier 1000-kernel weight were detected for P<sub>2</sub>, P<sub>4</sub>, P<sub>6</sub>, P<sub>1</sub>xP<sub>6</sub>, P<sub>2</sub>xP<sub>4</sub>, P<sub>2</sub>xP<sub>6</sub>, P<sub>2</sub>xP<sub>8</sub>, P<sub>4</sub>xP<sub>5</sub>, P<sub>4</sub>xP<sub>8</sub>, P<sub>5</sub>xP<sub>8</sub>, P<sub>6</sub>xP<sub>7</sub> and P<sub>6</sub>xP<sub>8</sub>. The parental No 6 (P<sub>6</sub>) gave the highest mean

value for biological yield/ plant and ranked the first parents for this traits. Moreover, the cross P1xP6 exhibited the highest crosses for biological yield/ plant. Parent No 2 (P<sub>2</sub>) and the cross P2xP4 gave the highest mean values for grain yield / plant. Therefore, these crosses could be efficient for prospective wheat breeding programs aiming at improving wheat grain yield.

**Table 6. Mean performance of the genotypes for yield and its components over the studied environments.**

Genotypes	plant height	spike length	No. of spikes /plant	1000 kernel weight	Biological yield/ plant	Grain yield/ plant
P1	70.13	11.25	29.25	38.3	207.5	16.5
P2	95.92	12.92	24.5	50.58	211.17	35.25
P3	79.67	12.08	28.42	35.48	134.92	16.75
P4	80.54	12.08	32.58	46.17	188.25	24.88
P5	75.17	11.42	17.33	40.37	128.67	16.5
P6	88.75	11.92	28.83	49.23	214.33	25.42
P7	82.17	9.33	29.67	39.37	166.5	26.67
P8	83.58	11.17	33.33	39.95	199.17	23.5
1x2	73.67	11.33	16.5	38.77	140.83	18.78
1x3	72.5	9.83	30.83	35.36	167	18.58
1x4	81	12.25	32.33	40.43	192.33	25.33
1x5	86.63	11.08	28.5	37	146.17	16.67
1x6	85.75	11.92	32.17	47.65	229.17	33
1x7	76.46	10.17	28.17	37.5	149.83	27.71
1x8	75.58	10.75	23.33	38.2	123	26.33
2x3	79.5	13.33	22.33	40.53	150	21.67
2x4	92.54	12.58	14.5	50.68	115.33	37.5
2x5	78.17	11.25	25.67	42.72	187.17	22.92
2x6	89.25	12.25	17.33	49.65	209.33	30.6
2x7	91.42	11.33	26.83	41.77	174.67	19.49
2x8	87.85	12.33	21.75	46.1	174.78	25.5
3x4	78.5	13	23.17	42.13	169.17	20.58
3x5	75.92	11.83	24.83	37.93	140	12.33
3x6	82.25	11.83	29.83	41.75	202.83	20.58
3x7	90.75	12.67	22.83	44.28	181.67	27.33
3x8	79.58	11	25.08	39.48	135.5	33.83
4x5	92.33	11.92	25.67	49.2	169.67	21.83
4x6	89.67	12.58	25.58	47.92	201	27.33
4x7	86.42	12.92	28.67	44.83	174.17	26.96
4x8	95.67	12.83	19	45.8	154.67	34.83
5x6	83.42	12.08	23	44.05	159.17	25.21
5x7	82.5	10.58	20.75	42.63	129.67	20.5
5x8	81.5	10.25	27.5	47.38	155.67	27.17
6x7	97.75	11.33	25.5	46.45	204.33	31
6x8	90.42	13.17	23.33	45.82	166.33	30.79
7x8	89.96	12.58	14.25	43.27	90.83	16.25
mean of parent	81.99	11.52	27.99	42.43	181.31	23.18
mean of cross	84.53	11.82	24.26	43.19	164.08	25.02
mean of Genotype	83.97	11.75	25.09	43.02	167.91	24.61
L.S.D 5%	8.14	1.78	3.59	5.68	14.22	5.52
L.S.D 1%	10.67	2.34	4.71	7.45	18.65	7.24



## Combining ability

The analysis of variance for combining ability for plant height, spike length, number of spikes/ plant, 1000-kernel weight, biological yield, and grain yield/ plant, under drought treatment, normal irrigation and combined analysis is presented in Table 6.

**Table (7) Combining abilities mean squares for yield and its components under normal irrigation and drought stress condition as well as the combined over them.**

S.O.V.	df	plant height	spike length	No. of spikes /plant	1000 kernel weight	Biological yield/ plant	Grain yield/ plant
<b>Drought environment</b>							
GCA	7	146.79**	1.68	46.40**	58.49**	3615.79**	94.33**
SCA	28	31.56**	0.64	31.24**	8.76**	782.02**	35.50**
Error	70	9.4	0.43	1.83	5.34	25.86	3.08
GCA/SCA		4.65	2.63	1.49	6.68	4.62	2.66
<b>Normal environment</b>							
GCA	7	158.34**	3.09**	22.91**	88.19**	2226.62**	121.27**
SCA	28	34.04**	1.1	41.88**	7.18**	1394.78**	44.53**
Error	70	7.85	0.39	1.53	3.06	26.77	4.87
GCA/SCA		4.65	2.81	0.55	12.29	1.6	2.72
<b>Combined analysis</b>							
GCA	7	277.47**	4.50**	60.46**	143.88**	4199.35**	162.32**
SCA	28	56.46**	1.25**	49.71**	12.96**	1542.31**	55.40**
GCA x L	7	27.67**	0.27	8.85**	2.8	1643.06**	53.27**
SCA x L	28	9.14	0.49	23.41**	2.98	634.50**	24.63**
Error	140	8.62	0.41	1.68	4.2	26.32	3.97
GCA/SCA		4.91	3.6	1.22	11.1	2.72	2.93
GCA x L/GCA		0.10	0.06	0.15	0.02	0.39	0.33
SCA x L/SCA		0.16	0.39	0.47	0.23	0.41	0.44

\* p< 0.05; \*\* p< 0.01

General (GCA) and specific (SCA) combining ability mean squares were highly significant for all studied traits in both environments as well as combined analysis except for spike length under drought and normal conditions. Such results indicated that both types of combining ability are important in the inheritance of these traits. Moreover, the ratios between GCA and SCA exceeded the unity for all studied traits, revealing that additive and additive x additive types of gene action are more important than non-additive gene action in controlling these traits. The genetic variance was previously reported to be mostly due to additive effects for plant height by **Menshawy (2004)** and **El Hosary et al (2009)**; for spikes/ plant by **El Seidy and Hamada (1997)**, **El Borhamy (2000)**, **Gomaa et al (2014)**; for 1000-grain weight by **El Seidy and Hamada (1997)**, **El Borhamy (2000)**, and for grain yield/ plant by **El Seidy and Hamada (1997)**, **El Seidy and Hamada (2000)**, **El Borhamy (2000)**, **Abd El-Aty and Katta (2002)**, **El Hosary et al (2012)**, **Gomaa et al (2014)**.

The mean squares of the interaction between GCA, SCA and irrigation treatments were significant for all studied traits except both types of combining abilities x E for spike length and 1000-kernel weight. Such result indicated that the additive and non-additive types of gene action differed significantly from one environment to another for these traits. Similar results were reported by **El-Seidy and Hamada (1997)**, **El-Seidy and Hamada (2000)**.

The ratio SCA x environment/ SCA was much higher than that of GCA x irrigation/ GCA treatments for all traits indicating that non-additive effects were much more influenced by environments than additive genetic one. Such results are in harmony with those obtained by **El Hosary and Nour El Deen (2015)**.

**General combining ability (GCA) effects:** Test of homogeneity revealed the validity of the combined analysis for the data of the two irrigation treatments. The general combining ability

effects  $\hat{g}_i$  of each parent for all studied measurements at the combined analysis are presented in Table (7).

**Table 8. Estimates of general combining ability effects for yield and its components at the combined analysis.**

Parent	plant height	Spike length	No of spike/ plant	1000-kernel weight	biological yield/ plant	grain yield / plant
P1	-6.39**	-0.60**	2.45**	-3.57**	5.21**	-2.21**
P2	2.85**	0.45**	-3.19**	2.42**	6.33**	2.54**
P3	-3.74**	0.19*	1.00**	-3.47**	-9.52**	-3.31**
P4	2.15**	0.65**	0.83**	2.62**	4.16**	2.26**
P5	-2.49**	-0.40**	-1.52**	-0.55*	-16.64**	-4.19**
P6	4.03**	0.32**	0.86**	3.46**	28.96**	2.78**
P7	2.39**	-0.55**	0.05	-0.77**	-7.30**	0.11
P8	1.20**	-0.05	-0.49**	-0.12	-11.21**	2.02**
L.S.D(0.05) gi	0.68	0.15	0.3	0.47	1.18	0.46
L.S.D(0.01) gi	0.89	0.19	0.39	0.62	1.55	0.6
L.S.D(0.05) gi-gj	1.29	0.28	0.57	0.9	2.25	0.87
L.S.D(0.01) gi-gj	1.69	0.37	0.74	1.18	2.95	1.15

\*  $p < 0.05$ ; \*\*  $p < 0.01$

Such results are being used to compare the average performance of each parent with other genotype and facilitate selection of parents for further improvement to drought resistance. Results indicate that the parental P<sub>1</sub> gave desirable significant  $\hat{g}_i$  effects for plant height, no of spike/ plant and biological yield/ plant. P<sub>2</sub> exhibited significant and positive  $\hat{g}_i$  effects for plant height, spike length, 1000-kernel weight, biological yield/ plant and grain yield/ plant. P<sub>3</sub> gave useful significant  $\hat{g}_i$  effects for plant height, spike length and no of spikes/ plant. P<sub>4</sub> expressed significant and positive  $\hat{g}_i$  effects for plant height, spike length, no of spikes/ plant, 1000-kernel weight, biological yield/ plant and grain yield/ plant. P<sub>5</sub> seemed good general combiner for plant height and grain. P<sub>6</sub> exhibited positive and significant  $\hat{g}_i$  effects spike length, no of spikes/ plant, 1000-grain, biological yield/ plant and grain yield/ plant. Also, it is considered the best combiner for grain yield/ plant and most of its components. P<sub>7</sub> and P<sub>8</sub> gave positive and significant combiner for plant height.

#### Specific combining ability (SCA) effects

Specific combining ability effects  $\hat{s}_{ij}$  for the F<sub>1</sub> crosses for the studied traits in the combined analysis are presented in (Table 8).

For plant height, six crosses expressed significant and positive  $\hat{s}_{ij}$  effects. Moreover, the cross P<sub>1</sub> x P<sub>5</sub> gave the most desirable  $\hat{s}_{ij}$  effects for plant height. However, three cross combinations i.e. P<sub>1</sub>xP<sub>2</sub>, P<sub>2</sub>xP<sub>5</sub> and P<sub>3</sub>xP<sub>4</sub> gave significant and negative  $\hat{s}_{ij}$  effects for the mention trait. For spike length, five crosses in the combined analysis expressed significant and positive  $\hat{s}_{ij}$  effects. Moreover, the cross P<sub>7</sub> x P<sub>8</sub> gave the most desirable  $\hat{s}_{ij}$  effects for this trait. For number of spikes/ plant, nine crosses expressed significant and positive  $\hat{s}_{ij}$  effects. However, the best  $\hat{s}_{ij}$  effects (5.29\*\*) were detected for the cross P<sub>2</sub> x P<sub>5</sub>. Regarding 1000-kernel weight, five cross combinations expressed significant and positive  $\hat{s}_{ij}$  effects. The cross P<sub>3</sub>xP<sub>7</sub> being the highest one in this traits and recorded 5.51\*\*. twelve crosses combinations exhibited significant and positive  $\hat{s}_{ij}$  effects for biological yield/ plant. The best positive  $\hat{s}_{ij}$  effects were the crosses P<sub>2</sub> x P<sub>5</sub> and P<sub>3</sub> x P<sub>7</sub> in the combined analysis (Table 8). Regarding to grain yield/ plant eight crosses i. e. P<sub>1</sub>xP<sub>6</sub>, P<sub>1</sub>xP<sub>7</sub>, P<sub>2</sub>xP<sub>4</sub>, P<sub>3</sub>xP<sub>7</sub>, P<sub>3</sub>xP<sub>8</sub>, P<sub>4</sub>xP<sub>8</sub>, P<sub>5</sub>xP<sub>8</sub> and P<sub>6</sub>xP<sub>7</sub> exhibited significant and positive  $\hat{s}_{ij}$  effects.

**Table 9. Estimates of specific combining ability effects for yield and its components 'at the combined analysis.**

cross combinations	plant height	Spike length	No of spike/ plant	1000-kernel weight	biological yield/ plant	grain yield / plant
P1xP2	-6.77**	-0.27	-7.85**	-3.11*	-38.62**	-6.16**
P1xP3	-1.34	-1.51**	2.30**	-0.62	3.4	-0.5
P1xP4	1.27	0.45	3.96**	-1.63	15.05**	0.67
P1xP5	11.54**	0.32	2.48**	-1.9	-10.32**	-1.54
P1xP6	4.14*	0.44	3.76**	4.74**	27.08**	7.81**
P1xP7	-3.51	-0.44	0.57	-1.18	-15.99**	5.20**
P1xP8	-3.2	-0.35	-3.72**	-1.13	-38.92**	1.91
P2xP3	-3.58	0.95*	-0.56	-1.44	-14.72**	-2.17
P2xP4	3.57	-0.26	-8.23**	2.63*	-63.07**	8.08**
P2xP5	-6.16**	-0.55	5.29**	-2.17	29.57**	-0.05
P2xP6	-1.6	-0.27	-5.43**	0.75	6.13	0.66
P2xP7	2.21	-0.31	4.88**	-2.90*	7.73*	-7.77**
P2xP8	-0.17	0.19	0.34	0.78	11.75**	-3.6**
P3xP4	-3.88*	0.41	-3.75**	-0.03	6.61*	-2.98*
P3xP5	-1.82	0.29	0.27	-1.06	-1.75	-4.78**
P3xP6	-2.01	-0.43	2.89**	-1.25	15.48**	-3.50**
P3xP7	8.13**	1.28**	-3.30**	5.51**	30.58**	5.92**
P3xP8	-1.85	-0.89*	-0.51	0.06	-11.68**	10.51**
P4xP5	8.71**	-0.09	1.27	4.12**	14.23**	-0.85
P4xP6	-0.48	-0.14	-1.2	-1.18	-0.04	-2.32
P4xP7	-2.09	1.07**	2.70**	-0.03	9.40**	-0.02
P4xP8	8.35**	0.49	-6.43**	0.29	-6.2	5.94**
P5xP6	-2.09	0.4	-1.43	-1.87	-21.07**	2.01
P5xP7	-1.36	-0.22	-2.87**	0.94	-14.30**	-0.02
P5xP8	-1.18	-1.05*	4.42**	5.04**	15.60**	4.72**
P6xP7	7.37**	-0.19	-0.5	0.74	14.76**	3.49**
P6xP8	1.22	1.15**	-2.13*	-0.54	-19.33**	1.37
P7xP8	2.4	1.44**	-10.40**	1.14	-58.57**	-10.48**
LSD5%(sij)	3.69	0.81	1.63	2.58	6.45	2.5
LSD1%(sij)	4.84	1.06	2.14	3.38	8.45	3.28
LSD5%(sij-sik)	5.46	1.2	2.41	3.81	9.54	3.7
LSD1%(sij-sik)	7.16	1.57	3.16	5	12.51	4.85
LSD5%(sij-skL)	1.82	0.4	0.8	1.27	3.18	1.23
LSD1%(sij-skL)	2.39	0.52	1.05	1.67	4.17	1.61

\* p< 0.05; \*\* p< 0.01

It could be concluded that the previous cross combinations might be of interest in breeding programs towards the development of pure lines varieties for high biological, and grain yields/ plant under drought conditions.

**The correlation between genetic distance and each of mean performance, heterosis and SCA for grain yield/plant:** The correlation of GD and each of SCA and heterosis for grain yield for 28 hybrids combination are estimated. The estimated values of correlation coefficient between GD, and each of mean performance and heterosis relative to Mp as well as Bp and SCA for grain yield/plant found positive (r = 0.134, 0.067, 0.035 and 0.238, respectively) Table (10). Therefore, specified

tendency could be predicted about the relationship of GD and heterosis for grain yield/plant in this study. **El-Maghraby et al. (2005)** reported that ISSRs are useful in detecting a high level of polymorphism among wheat cultivars. **Also, Prasad et al. (2000)** illustrated that the utility of ISSR markers for detecting polymorphism leading to genotype identification and for estimating genetic diversity.

**Table 10. Genetic diversity, mean performance, heterosis, heterobiltosis and specific combining ability for grain yield for all studied crosses.**

Crosses	genetic diversity	grain yield			
		mean performance	Heterosis		SCA
			Mp	BP	
1x2	0.25	18.78	-27.43	-46.73	-6.16
1x3	0.26	18.58	11.78	10.95	-0.5
1x4	0.235	25.33	22.46	1.84	0.67
1x5	0.3	16.67	1.01	1.01	-1.54
1x6	0.24	33	57.46	29.84	7.81
1x7	0.353	27.71	28.38	3.91	5.2
1x8	0.314	26.33	31.67	12.06	1.91
2x3	0.36	21.67	-16.67	-38.53	-2.17
2x4	0.3	37.5	24.74	6.38	8.08
2x5	0.298	22.92	-11.43	-34.99	-0.05
2x6	0.306	30.6	0.89	-13.18	0.66
2x7	0.319	19.49	-37.06	-44.72	-7.77
2x8	0.239	25.5	-13.19	-27.66	-3.6
3x4	0.116	20.58	-1.1	-17.25	-2.98
3x5	0.273	12.33	-25.81	-26.37	-4.78
3x6	0.319	20.58	-2.37	-19.02	-3.5
3x7	0.37	27.33	25.91	2.5	5.92
3x8	0.326	33.83	68.12	43.97	10.51
4x5	0.163	21.83	5.54	-12.23	-0.85
4x6	0.218	27.33	8.7	7.54	-2.32
4x7	0.267	26.96	4.61	1.09	-0.02
4x8	0.223	34.83	44.01	40.03	5.94
5x6	0.167	25.21	20.28	-0.82	2.01
5x7	0.175	20.5	-5.02	-23.13	-0.02
5x8	0.125	27.17	35.83	15.6	4.72
6x7	0.233	31	19.04	16.25	3.49
6x8	0.186	30.79	25.89	21.15	1.37
7x8	0.103	16.25	-35.22	-39.06	-10.48

The results indicated that ISSR marker can be used as a tool for determining the extent of genetic diversity among wheat genotypes and for genotypes into different groups. This study showed that GD can be used to precisely predict the yield performance and heterosis value for F<sub>1</sub> hybrids. For more accuracy the more primers must be used to detection the difference between parents or can use more markers.

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