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RAPD MARKERS FOR SALINITY TOLERANCE IN BREAD WHEAT (*Triticum aestivum*)

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ABSTRACT

Ten F_4 families with the highest grain yield/plant were selected from cross between two cultivars (long spike-58 × Giza -168) and evaluated for tolerance to salinity stress conditions. Crosses were also made between ten selected plants. In the second cycle of selection, F_5 families were classified into two groups according to their performance of some salinity stress related traits such as grain yield and stress physiological traits. Significant positive response to selection for grain yield was ranged from 69.94% of means to 135.83% for intra cross between selections within population. The indirect response to selection ranged from 108.34% for proline content to 42.34% in chlorophyll content. The results indicated the presence of three positive and four negative RAPD markers that could be considered as reliable markers for salinity tolerance in bread wheat.

INTRODUCTION

Wheat is the most important cereal crop grown in the world and it is the most cereal crop in Egypt. Wheat is a staple food in Egypt. Increasing wheat yield per unit area can be achieved by breeding high yielding varieties. Wheat production is limited mainly by the availability water resources and soil salinity. Salinity is a major factor limiting plant growth and leads to lower agricultural production in arid and semi-arid region (Bai *et al*., 2011). High soil salinity is one of the important environmental factors that limit distribution and productivity of major crops (Chandan *et al*., 2006).

Agricultural productivity in arid and semiarid regions of the world is very low due to accumulation of salt in soils (Ashraf and Sarwar 2002 and Munns; 2002). Wheat is a moderately salt tolerant crop (Khan *et al.,* 2004) and for screening or developing salt tolerant wheat varieties, biochemical studies are necessary to identify the physiological and biochemical markers. By using these markers available wheat germplasm can be screened for salt tolerance or by incorporating them into new high yielding salt tolerant wheat varieties. It was estimated that 20% of the irrigated land in the world is affected by salinity (Yamaguchi and Blumwald 2005)..

Wheat is also a crop in which there is variation for salinity tolerance which may be used for improving salinity tolerance (Ashraf and Meneily 1988).

Wheat genotypes with higher proline, K/Na ratio and chlorophyll contents had higher grain yield. On the basis of yield reduction, three genotypes viz, Lu-26s, Sars abz and KTDH were found tolerant. These genotypes also maintained the higher concentration of proline, K/Na ratio and chlorophyll contents under saline conditions (Khan *et al.,* 2009).

Shamsi Keyvan (2011) found that with an increase in the intensity of drought stress on wheat cultivars, there was a decrease in relative water content,

total chlorophyll and increased proline content Ranjbar *et al*., (2010) showed that grain yield and kernel per spike under non-saline conditions were significantly, higher than these under saline conditions for all genotypes. Genotype No. 5 (pf 70354/Bow) produced the highest grain yield in saline and non – saline conditions by producing 723.44 and 976.56 gm2.

Salinity increased Na^+, decreased 1000 grain weight and biological yield decreased with increasing salinity.Number of tillers panicle length and grain yield decreased similarly by the salinity of 6 and 10 ds/ml (Islam *et al.,* 2011).The salt tolerance abilities of landraces have been evaluated by many workers (Rana 1986 and Martin *et al*., 1994).Salinity reduced performance with regard to grain yield/plant, 1000 grain weight, number of grain and spike length (Sadat et al., 2006).Also, salinity reduced number of grains which was mainly found responsible for reduction in grain yield, Generally, genotypes having ability to exclude not from shoot were found salt tolerance in respect of grain yield (Abid *et al*., 2009). Significant correlation between chlorophyll content and grain yield under heat and drought stress can contribute to decrease drought intensity damage due to reduction of chlorophyll content through light absorption (Mohtasham *et al.,* 2009). Salt tolerance varieties produced more grain yield than the local variety by 15%. Grain yield of salt tolerance varieties were significantly correlated with number of kernel and biological yield. Based on these results, we propose that Bam, siston and Kavir could be considered as the new high yielding cultivars of wheat for salt affected area of the LKBB (Ranjbar *et al.,* 2010).

The observed gain from selection and heritability estimate point to kernel weight being controlled by several genes with small effects. Selection for increased kernel size resulted in increased flout yield (Jochum *et al*.,2001).Bread wheat (*Triticum aestivum L*.) is a major crop in most of the countries of the world with suffer saline soils. Because of its global importance as crop by for the greatest attention to selection and breeding for salinity tolerance has been given *Triticum aestivum*. Hybridization is a useful tool for creating genetic variation within the crop species to produce transgressive segregants. Hybridization of wheat for salt tolerance has involved crosses within species between intermediately tolerance accessions seeking transgressive segregants involving salt tolerant genes.In the present study, selections with intermating within population of crossing is used for seeking transgressive segregants involving salt tolerance genes. Crossing such selected genotypes maximize the chance of obtaining combination of genes which operate salt tolerance in selected genotypes yielding.The results of selection conducted for low and high values of yield components. Correlations between grain yield and yield components and heritability values revealed that the number of grains could be used as selection criteria (Halil and Neemi 2005).

Marker-assisted selection for qualitative appeared most successful after DNA fingerprinting, while for quantitative characters major disease resistance genes and genes controlling QTL for abiotic stress tolerance (Farooq and Azam 2002).

Marker-assisted selection (MAS) is becoming the method of choise in facilitating tagging of the desirable traits in many crops (Abdel-Tawab *et al*.,2003).

Five pairs of genome specific primers designed for wheat Orebi genes were used for DNA amplification. Two primer P_2 1F/P_2/R and P_2 5F/PR, amplified 596 and 1113bP fragments, respectively from the A genome. It was found out that Dreb l gene was located in chromosome 3A in all genotypes including drought tolerance and drought sensitive one, excepting semi- tolerance genotypes (Irada and Samira 2010).

Marker assisted selection (MAS) provides a strategy for accelerating the process of wheat breeding (Wei et al., 2009). Rashed *et al*.(2010) used two selected varieties, their F_1 and F_2 plants which were evaluated for their response to drought stress by recording some drought related traits. Five individual plants of the two contrasting F_2 plant group(the most tolerant and most sensitive group), the parents and their F_1 plants were used to develop some molecular genetic markers associated with drought tolerance in wheat by using nine RAPD primers. The results indicated that the presence of four positive and two negative RAPD markers that could be considered as reliable markers for drought tolerance in wheat. A yield increase of 15% was observed after two cycles of recurrent selection. The recurrent selection scheme employed in this study modified the character under selection allowing the identification of superior genotypes (Maich *et al*., 2000). The genetic variation and relationships among different wheat genotypes with different response to salt stress were also investigated by RAPD and SSR analysis. 82 out of 118 RAPD marker detected were polymorphic (69.5%) and 42 out of 59 SSR alleles were polymorphic (71%) and can be considered as useful. In this context, marker for the wheat cultivars tested. Seven markers distinguished Benesweif cultivar, six markers for the cultivar Sohag, and two markers for the cultivar Gemmiza 10.These markers can be verified as being genetic markers associated with salt tolerance in the three wheat genotypes and help in marker-assisted selection breeding program (Reda *et al*., 2011).

The objectives of the present study were as follow ; (i) determine the genetic gain for selection of grain yield and responses on other agronomic traits under salinity stress (ii) to investigate the variation in F_5 selected families with highest yield, F_5 selections with intermating within population, non selected F_5 families and the two local varieties with respect to salinity tolerance based on their performance for some salinity related trait and to detected some RAPD markers associated with salt tolerance to be used in marker-assisted selection (MAS) programs.

MATERIALS AND METHODS

Materials:

A field experiment was conducted over the two seasons of 2010 and 2011 at the Experimental Farm of south Valley University. The soil type is sandy loam, Sandy 74%, silt 16.6% and clay 9.4; pH 8.25; E.C 9.2 dsm^{-1}. The electric conduction for irrigation water 5.4 dsm^{-1}. Selection studies were initiated in the winter of 2010 in F_4 generation of the cross (long spike-58 x Giza-168).

Methods:

Field trials:

Ten F_4 families with highest yield under salinity stress, F_4 non selected families (bulk) and the two local varieties Giza-168 and Sids-12 were planted on 1st Dec 2010 in 3 row plot 3m in length with 20 cm row spacing. Within each family with highest yield one plant labeled and tagged. Crosses were made between the 10 selected plants (intermated) by pair crossing using a circular mating which yielded 5 crosses, were harvested and seeds per cross were bulked to form the intrapopulation cross (selection with intermating within population).

Selfed seeds were also harvest separately from each individual plant and were kept distinct form F_5 selected families.

Subsequent cycle was generated by intermating selected plants in F_4 family after growing in the field.

A total of 18 families were evaluated in next season on 5th Dec in 2011 season which comprised of 10 F_5 selected families, one progenies of the intrapopulation cross, five bulk families (non-selected families) and the two local varieties Giza-168 and Sids-12.

The experimental layout was a randomized complete block design with three replications. Each family was represented in each block by a single row containing 15 plants spaced 20 cm from each other.

Measurements:

Agronomic traits

Response variable included agronomic traits such as follow:

1- grain yield/ plant in gram (g) which related to drought stress (Fisher and Maurer, 1978).
2- 1000 grain weight in gram (g).
3- Number of kernel/spike (N).
4- Spike length (cm).

Biochemical traits:

Biochemical traits related to salinity stress were determined as follow:

1- proline content as estimated according to Bates *et al*.. (1973).
2- Chlorophyll content as measured by Sûkran *et al*. (1998).

Statistical analysis:

Response variables were subjected to analysis of variance (ANOVA) in the selection study (Snedecor and Cochran 1980). Differences between means were tested by the revised LSD according to (El-Rawi and Khala Falla 1980).

Correlation was computed according to (Miller et al., 1958). Heritability (H^2) = $\sigma^2g/\ \sigma^2p$ where σ^2g the genetic variance and σ^2p phenotypic variance (Mather and Jinks, 1971). The predicted response to selection (Rx) was estimated as $Rx=i.h^2.\ \sigma p$ where i= standardized selection differential, h^2= heritability, σ^2p= phenotypic standard deviation.

While, the indirect response to selection (CRx) was estimated as $CRx=ih^2\ \sigma p\ rxy$ where rxy is the genetic correlation between selected trait and unselected traits. Observed direct and indirect responses to selection were expressed as percent change in the population mean (falconer 1989)

At the end of 2011 season, F_5 families were classified into two groups according to their performance for some salinity stress related traits:

A- The first group as sensitive to salinity stress, comprised two F_5 bulk No 7 and 8 and Sids-12No 9.

B- The second group as tolerant to salinity stress which comprised four F_5 selected families No.1, No.2, No.3 and No.4 and two families with highest yield, highest proline content and chlorophyll content from the intra population cross were salt tolerance No 5 and No6.

RAPD PCR analysis:

DNA was extracted from leaves using the organs DNeasy (Qiagen santa clara, Ca) in the growth room 5-7 cut long piece of fresh leaf material was cut from the plants and the leaf tissues were ground. This was performed according to (Murray and Thompson ,1980 ; and Saghai- Maroof *et al*.,1984).

RAPD Reactions:

A set of ten primers RAPD (Table 7) was used. The amplification reaction PCR system 9700 programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94 °C.

Each cycle consisted of a denaturation step at 94 °C for 1 min an annealing step at 63 °C for 1 min and elongation step at 72 °C for 1.5 min, the primer extension segment was extended to 7 min at 72°C un the final cycle.

The amplification products were resolved by electophorasis in a 1.5 agarose gel containing ethidium bromide (0.5 mg/ml) in 1XTBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a polariod camera. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or as 0, respectively. PCR reactions were performed according to Williams *et al.,* (1990).

RESULTS AND DISCUSSION

Response to selection for grain yield per plant

The analysis of variance revealed that the differences in grain yield /plant among F_5 families were highly significant (Table 2). Significant positive response to selection for grain yield was obtained which ranged from 69.94% of family means to 135.83% for intra population crosses. Greater obtained response for grain yield was by the intra population crosses. Since the highest selected plants within population were intermated, the superiority of the intra population cross suggests that different yield genes expressed which were incorporated into the genotypes produced by intercrossing within population. The highest yield of F_5 selected families consistently displayed greater drought grain yield (Fisher and Maurer 1978). Greater response to selection contributed to significant correlation between chlorophyll content and grain yield under drought stress which decrease of intensity damage due to reduction of chlorophyll content through light absorption (Mohtasham *et al*., 2009).

The results showed that there was a decrease in relative water, total chlorophyll content and increased proline content with an increase in the intensity of drought stress on wheat cultivars (Shamsi Keyvan 2011).

Correlation between grain yield and chlorophyll and proline content was positive and significant being 0.34 and 0.35 (Table 6). Also, greater response to selection for grain yield with intra population crossing could reflect a higher selection intensity (1/15).

Selections in F_5 exceeded that Giza-168 by 2.34 (g) and Sids-12 by 2.42 (g) of mean . Salt tolerant varieties produced more grain yield than local variety by 15%. (Ranjbar *et al.,*2010).

Correlated response:

Agronomic parameters:

The differences between F5 families for 1000 grain weight and number of kernel/spike were highly significant (Table 2). The observed correlated response to selection ranged from 14.69% for 1000 grain weight to 48.83% for number of kernel/spike.

Our results are in agreement with those of Ranjbar *et al*.(2010) who found significantly correlated response with number of kernel and biological yield under drought stress.

Biochemical traits:

The data in Table 2 showed that the differences to salinity tolerance between F_5 families were significant for proline content which is considered a related trait. The positive correlated response to selection for grain yield in proline content was 108.34% with F_5 selected families. As to intra population crosses was 42.34%. While, the correlated response to selection for chlorophyll ranged from 15.26 for F_5 selected families to 33.15% with the intrapopulation cross. Significant correlation between chlorophyll content and grain yield under heat and drought stress can contribute to decrease drought intensity damage due to reduction of chlorophyll content through light absorption (Mohtasham *et al*., 2009)

Uniformally, the direct and indirect response to selection were greater than predicted response for all traits studied except 1000 grain weight indicated that dominance gene effects involved in the inheritance of that trait.

Mean performance under salinity stress

The mean performance of tolerant and sensitive F_5 families and the two local variety Sids-12 and Giza -168 are shown in Table 5.

The F_5 families were classified into two groups according to their behavior under Salinity stress (Table 5). Furthermore, six families representing the most salt tolerant genotypes and three families representing the most salt sensitive ones were selected on the basis of their performance with respect to grain yield, chlorophyll content and proline content. Comparisons between the means of the two groups regarding each trait indicated phenotypically marked differences between the two contrasting groups in F_5 generation.

Therefore, the F_5 selected plants were used as bulked segregants. The results agreed with Khan *et al*. (2009), who found four genotypes Viz, Lu-26s, Sarsabz and KTDH with the higher concentration of proline, K/Na ratio and chlorophyll contents and higher grain yield under saline conditions, as well as obtained a molecular markers associated with salt tolerance by using PCR-RAPD technique.

Salinity increased Na^+, decreased 1000 grain weight and biological yield decreased with increasing salinity. Number of tillers panicle length and grain yield decreased similarity by the salinity of 6 and 10 ds/m^{-1}(Islam *et al*., 2011).

T1-2-3

T4-5

Also, Sadat *et al*., (2006) showed that the salinity reduced performance with regard grain yield per plant 1000 grain weight, number of grains and spike length.

RAPD markers for salinity tolerance:

DNA isolated from F_5 selected families, F_5 intrapopulation crosses as a salinity tolerance, F_5 bulk families (non selected families) and Sids-12 as a salinity sensitive were tested against ten primers as shown in Figures1 and 2 and summarized in Table 7.

Table 6. Correlation between selected and unselected traits, heritabilities and genetic variance.

	Correlation	Heritability	Genetic variance
Grain yield	-	0.92	0.23
1000 grain weight	0.47*	0.67	74.24
Number of kernel	0.62*	0.69	118.73
Spike length	0.25	0.67	3.79
Proline content	0.34*	0.62	5.80
Chlorophyll	0.35*	0.61	901.9

Table 7. List of primers used and their nucleotides sequences.

Primer	Sequence	Primer	Sequence
E-01	/1CCCAAGGTCC-3-/	B-14	/1TCCGCTCTGG-3-/
A-07	/1GAAACGGGTG-3-/	C-12	/1TGTCATCCCC-3-/
A-12	/1TCGGCGATAG-3-/	G-05	/1CTGAGACGGA-3-/
A-02	/1TGCCGAGCTG-3-/	G-12	/1CAGCTCACGA-3-/
B-13	/1TTCCCCCGCT-3-/	H-15	/1AATGGCGCAG-3-/

Six primers only gave a polymorphism with the studied genotypes (Table 9) with three primers out of them developed molecular markers for salinity tolerance are illustrated in Table(8) namely, A7, C12 and B14.

A7, C12 and B14 primers exhibited three positive molecular markers with molecular size of 150bP for A7, 250 bp for C12 and 50 bp for B14 which were found only in tolerant F_5 selected families, while they were absent in the sensitive F_5 bulk families (non selected) and Sids-12 cultivar.

H15 and B13 primers exhibited four negative molecular markers with molecular size of 300bP, 1000 bp, 1100 bp for H15 and 750 bp for B13, which were found only in the sensitive F_5 unselected families and Sids-12, while they were absent in tolerant F_5 selected families.

These three positive and four negative RAPD markers could be considered reliable markers for salinity tolerance in wheat.

These results agreed with Reda *et al*.(2011) ,who used RAPD markers to detect DNA polymorphism in nine wheat genotypes. They found 82 out of 118 RAPD markers detected were polymorphic 69.5% and can be considered as useful markers for the wheat cultivars tested. 18 random amplified polymorphic DNAs (RAPD) markers generated were found to be genotype specific.

Table 8. Survey of the ten primers fragments with F_5 selected, F_5 intra cross, F_5 non selected and Sids-12.

Primer name	MS Pb	T_1	T_2	T_3	T_4	T_5	T_6	S_1	S_2	S_3	M.T
A7	150	1	1	1	1	1	1	1	0	0	P
	250	1	1	1	1	1	1	1	1	1	-
C12	250	1	0	0	1	1	1	0	0	0	P
	350	1	1	1	1	1	1	1	1	1	-
A12	50	1	1	1	1	1	1	1	1	1	-
	100	1	1	1	1	1	1	1	1	0	-
B14	50	1	1	1	1	1	1	1	0	0	P
	100	1	1	1	1	1	1	1	1	1	-
G12	250	1	1	1	1	1	1	1	1	1	-
	400	0	0	1	0	0	1	0	0	0	-
	650	1	1	0	1	1	1	1	1	1	-
	750	0	0	0	0	1	1	0	0	1	-
	800	0	0	0	0	1	1	0	0	1	-
H15	300	0	0	1	0	0	1	1	1	1	N
	350	1	1	1	1	1	1	1	1	1	-
	1000	1	0	0	0	0	1	1	1	1	N
	1100	1	0	0	0	0	1	1	1	1	N
A2	100	1	1	1	1	1	1	1	1	0	-
	450	1	1	1	1	1	0	1	1	1	-
B13	200	1	1	1	1	0	1	1	0	0	-
	750	0	0	0	0	0	0	1	1	0	N
E1	100	1	1	1	1	1	1	1	1	1	-
	250	1	1	1	1	1	1	1	1	1	-
G5	100	1	1	1	1	1	1	1	1	1	-
	200	1	1	1	1	1	1	1	1	1	-

T = tolerant F_5 selected plants. **S = sensitive F_5 unselected and Sids-12**
Ms = Molecular size **P = positive** **N = negative** **MT = marker type**

Table 9. Polymorphism percentage generated by the ten primers in the wheat genotypes.

Primer	Monomorphic bands	polymorphic bands		Total bands	Polymorphic %
		Unique	Non unique		
A7	4	0	1	5	20
B13	6	0	2	8	25
C12	4	0	1	5	20
A12	9	1	0	10	0
B14	6	1	1	8	43
G12	6	1	3	10	30
A2	1	4	0	5	0
H15	2	2	3	7	42
E1	8	0	0	8	0
G5	5	0	0	5	0

Fig 1. RAPD PCR fragments of three primers [A7 (upper), C12(middle), B14(lower)] for the most tolerant F_5 plants selected, F_5 sensitive plant (bulk) and the sensitive parent sids-12.

Fig 2. RAPD PCR fragments of three primers[B13(upper), H15(middle), G12(lower)] for the most tolerant F_5 plants selected, F_5 sensitive plant (bulk) and the sensitive parent sids-12.

Fig3. RAPD PCR fragments of four primers [E1(upper), G5(middle), A12, A2(lower)] for the most tolerant F_5 plants selected, F_5 sensitive plant (bulk) and the sensitive parent sids-12.

Similar results were obtained by Abdel- Tawb *et al*., (1997) who detected five positive and negative RAPD markers for drought tolerance in Egyptian bread wheat. Nachit *et al.,* (2000) , found that yield related traits as grain yield, yield components and stress physiological traits were associated with some molecular markers in durum wheat.Several markers showed strong relationship with grain yield components and stress physiological traits with some molecular markers in durum wheat.Several markers showed strong relationship with grain yield components and stress physiological traits indicating that there are potential markers could be used as a markers assisted selection to improve abiotic stresses tolerance by molecular breeding our results are in agreement with those obtained by Rashed *et al* ., (2010) , who found the presence of four positive and two negative RAPD markers that could be considered as reliable markers for drought tolerance in wheat.The bulked segregant analysis was used in the random amplified polymorphic DNA (RAPD) technique. DNA polymorphisms were observed using 148 primers. The primer OPZ-10 amplified a 680 bp polymorphic DNA fragment which linked to K- no ratio trait (Mehboob *et al*., 2004).Only, three primers revealed polymorphism and developed molecular markers for salinity as clearly shown by the grain yield, proline content and chlorophyll content (Table 5).

The highest percentage of polymorphic bands among all tested genotypes was 42% for H15 primer and the lowest polymorphic band was 13% for B14 primer.RAPD banding patterns for the six wheat genotypes by using six primers scored three negative and one positive molecular markers correlated to the relatively sensitive wheat genotypes and three positive molecular markers which appeared in the tolerant genotypes (Mar-5 and Gem-7).Also,UBC78 operon primer differentiates the highest salt tolerant genotype (Mar-5) by the positive unique band of (110 bp) Samy *et al.,(*2007)

Conclusion:

In conclusion, the results of this investigation provided some PCR-RAPD based molecular marker associated either positively or negatively with wheat genotypes productivity this could be used to enhance breeding programs to improve their tolerance by pyramiding genes controlling this polygenic character by the aid of marker assisted selection. It is feasible that more markers can be generated for salt tolerance if more random primers were used.

At least, the RAPD markers developed from this study can be used in any further study to identify salt-tolerant genotypes in wheat.

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الواسمات الجزيئية المرتبطة بالانتخاب لتحمل الملوحة فى القمح المصرى

ثـروت محمـد الأمـين [1]، عبيـد محمـد أحمـد إبـراهيم [1] ، مجـدى عبـد الراضـى السـيد [2] و إبراهيم بيومى عبد الفريض [2]

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تم إنتخاب وزراعة عدد عشرة عائلات عالية المحصول من عشيرة إنعزالية من نبات القمح (Giza 168X Long spike 58) فى الجيل الرا بع فى أرض ملحية بمزرعة كلية الزراعة جامعة جنوب الوادى. أجرى التهجين بين العشرة عائلات المنتخبة بنظام التهجين الزوجى ثم خلطت البذور الناتجة من هذا التهجين للحصول على بذور Intrapopulation cross . تم تقسيم عائلات الجيل الخامس فى الدورة الثانية من الانتخاب الى مجموعتين:المجموعة الاولى تتحمل الملوحة وهى العائلات رقم 1.2.3.4.5.6، والمجموعة الثانية حساسة للملوحة وهى العائلات رقم 7.8. والصنف المحلى سدس 12، وذلك بناء على أدائها لصفة محصول الحبوب تحت الظروف البيئية المالحة .أظهرت النتائج أن الاستجابة للإنتخاب لمحصول الحبوب كانت موجبة ومعنوية حيث تراوحت من 64.94% للإنتخاب العائلى الى 135.83% للإنتخاب مع التهجين بين المنتخبات داخل العائلة، كما أوضحت النتائج وجود ثلاث واسمات جزيئية وراثية موجبة لتحمل الملوحة فى القمح ،وأربع واسمات جزيئية وراثية سالبة لتحمل الملوحة فى القمح.عكست النتائج النهائية أنة من الواضح أن السلالات أرقام 1.2.3.4.5.6 تتحمل الملوحة وقد ظهر بها الواسمات الجزيئية الوراثية الثلاث ذات الوزن الجزئى 150 . 250 . 50 .

قام بتحكيم البحث

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Table 1. Direct and indirect response to selection for grain yield in F_5 generation.

Cycle	Grain yield			1000 grain weight			Number of kernel			Spike length			Chlorophyll content			proline		
	Mean	O%	P%	Mean	CR%	P%	Mean	CR%	P%	Mean	CR%	P%	Mean	CR%	P%	Mean	CR%	P%
F_5 bulk	1.73			41.64			42.14			11.23			212.63			14.64		
Giza-168	2.04			37.25			55			12.0			211.23			15.3		
Sids-12	1.66			35.18			47			12.0			209.73			14.76		
F_5 selected families	2.94	69.94	0.87	47.76	14.69	13.50	62.72	48.83	10.71	14.06	25.2	0.76	245.08	15.26	21.37	30.56	108.34	11.00
F_5 intra population cross	4.08	135.83		45.45	9.14		89.0	110.19		15.0	33.57		283.12	33.15		20.84	42.34	

O % observed response ,P% predicted response ,CR % correlated response

Table 2. Pertinent Ms of the different items of the analyses of variance for agronomic traits and biochemical traits.

Item	Grain yield	1000 grain weight	Number of kernel	Spike length	Proline content	Chlorophyll content
Among F_5 families	1.81**	93.96**	719.46**	10.34**	737.90*	2557.91
Among F_5 selected	1.04**	45.86	576.98**	3.98**	684.41*	3175.01
Among F_5 bulk	0.24*	110.28*	170.54*	5.64*	15.38	9765.24**
F_5 selected Vs F_5 bulk	15.23**	480.67**	5415.68**	102.06**	231.93	10530.74
F_5 intra population cross Vs F5 bulk	13.86**	38.06	57.59.33**	51.34**	79.12	13970.04
Error	0.14	39.67	95.72	1.38	223.98	1656.82

***,** significant at 0.05, and 0.01 levels of propability ,respectively .**

Table 3. Cycle means of agronomic traits following of selection for grain yield.

Cycle	Grain yield	1000 grain weight	Number of kernel	Spike length	Proline content	Chlorophyll content
C0	2.67	47.29	56.50	11.50	-	-
C1-S	4.08	45.45	89.33	15.00	20.84	283.08
C1	2.95	47.76	62.72	14.06	33.69	245.08

C0 = base population
C1-s = selection with intermating within population
C1 = selection with selfing

Table 4. Agronomic traits, grain yield, chlorophyll content and praline content of 18 families of bread wheat genotypes.

Entry No	Family	Grain yield	Chlorophyll content	Proline	1000 grain weight	Number of kernel	Spike length
1	F_{5s}	2.70	202.32	18.34	50.88	53	14
2	F_{5s}	2.91	239.56	17.82	53.98	56	16
3	F_{5s}	2.46	216.71	49.39	45.38	55	12
4	F_{5s}	2.21	250.29	26.09	47.02	47	14
5	F_{5s}	3.04	263.17	55.53	49.08	62	14
6	F_{5s}	3.21	258.75	57.34	44.86	72	14
7	F_{5s}	2.71	237.18	41.95	40.45	68	14
8	F_{5s}	2.99	273.78	37.06	53.18	56	13
9	F_{5s}	3.83	260.82	39.26	47.56	83	15
10	F_{5s}	2.28	289.8	22.62	47.50	48	13
11	F_5 cross	4.08	283.08	20.84	45.45	89	15
12	F_5 bulk	1.39	292.47	11.27	39.93	35	10
13	F_5 bulk	1.70	131.57	18.84	43.67	39	13
14	F_5 bulk	2.03	213.87	14.03	53.79	38	10
15	F_5 bulk	1.36	212.63	14.65	39.51	35	10
16	F_5 bulk	1.70	211.90	14.00	42.16	44	11
17	Giza-168	2.04	211.23	15.30	37.25	55	12
18	Sids-12	1.66	209.73	14.76	35.18	47	12

F_5 = selected families, F_5 bulk = unselcted families
F_5 cross = intra population cross .

Table 5. Mean performance of tolerant and sensitive F_5 families and the two local varieties at the end of the experiment.

Traits	Family	No	Proline	Chlorophyll	Grain yield	1000 grain weight	Number of kernel	Spike length
Tolerant	F_5 selected families	3 T_1	49.39	216.71	2.46	45.38	55	12
		5 T_2	55.53	263.17	3.04	49.08	62	14
		6 T_3	57.34	258.75	3.21	44.86	72	14
		9 T_4	39.26	260.82	3.83	47.56	83	15
	F5 intra population cross	1 T_5	17.44	258.85	3.76	44.76	84	16
		2 T_6	24.25	307.30	4.08	49.7	100	15
Sensitive	F_5 bulk	1 S1	11.27*	292.47	1.21	37.81	32	9
	F_5 bulk	1 S2	18.64	213.87	1.68	42.00	40	10
	Sids-12	1 S3	15.30	211.23	2.04	37.25	55	12
L.S.D 0.05			29.93	85.29	0.89	8.33	19.03	1.04