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DIALLEL STUDY ON SOME *in vitro* CALLUS TRAITS OF BREAD WHEAT (*Triticum aestivum* L.) under salt stress

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ABSTRACT

Combining ability and heterosis were performed for *in vitro* traits in a diallel crosses involving six bread wheat genotypes under three levels of salt stress (0, 4000 and 12000 ppm). Mean squares of genotypes, parents and resulted fifteen hybrid combinations were found to be highly significant for most *in vitro* studied traits. Mean square estimates of parent vs. crosses were found to be highly significant for most studied traits. Both general (GCA) and specific combining ability (SCA) variances were found to be highly significant for most *in vitro* studied traits. Furthermore, the GCA/SCA ratios were found to be high than unity for most studied traits. The Egyptian wheat genotypes Gemmiza 1 and Giza 157 were considered to be good general combiner for most studied traits. The present study indicates that *in vitro* traits could be successfully used in genetic characterization for salt tolerance in bread wheat. Also, Information generated from this study can be used to select parents and hybrids forsalt tolerance in bread wheat breeding program.

Keywords: Wheat (*Triticum aestivum* L.), Diallel cross, Heterosis, Combining ability, *in vitro* traits, Salt tolerance.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the most important and first strategic cereal crop in Egypt. Also, bread wheat considered as a fundamental staple food crop for more than roughly 33% of the world population and the chief food for Egypt. There is a deficiency of wheat production in Egypt **(El-Sayed *et al.*, 2016)**. More than 50% wheat is imported for yearly consumption. Due to

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great increase in population, Egypt needs to increase its wheat production through its cultivation in the new reclaimed soils especially under saline conditions **(Salam 2002)**. Salinity is a main factor limiting crop productivity in arid and semi-arid areas of the world **(Ashraf, 1994; Hollington, 1998)**. Also, bread wheat widely cultivated in most of countries of the world which suffer saline soils and therefore increasing salt tolerance in wheat is necessary (**Tuna *et al*., 2008**). For this reason, the development of salt tolerance bread wheat genotypes is important (**Flowers *et al*., 1997, Ma *et al*., 2007, Diaz De Leon *et al*., 2011 and Salem and Mattar, 2014**). In bread wheat germplasm, salt is one of the major abiotic stress factor reducing plant growth and crop productivity (**Diaz De Leon *et al*., 2000**). To obtain better yield from saline soils and saline irrigation waters on a sustained basis, it is imperative that along with improved agronomic practices. *In vitro* culture of plant tissues has been a useful tool to study salt tolerance mechanisms at the plant cell level. Plant cell culture technology has potential application for selecting cell tolerant to salts in the culture medium and from these tolerant cells regenerate plants which are more resistant to the salts than parental materials **(Abdel-Hady 1999, Hala *et al.,* 2012, Salma *et al.,* 2013 and Mona Ismail 2014).** Many laboratories have reported on the selection of NaCl tolerant callus lines **(Yang *et al.,* 1990)**.

In order to improve productivity, one of the most important steps in a breeding program is the choice of suitable parents. To achieve gains in the plant biotechnology of wheat using immature embryo culture system, combining abilities for *in vitro* traits is necessary under salt stress (**Emara *et al*., 2013**). Several studies of the genetic control of callus formation and plant regeneration using immature embryo were also reported in maize (**williaman *et al*., 1988**), rice (**Peny and Hodgeo,1989, ABE and Futsuhara, 1991, Emara *et al*., 2013**) and wheat (**Qu *et al*., 1989, Barakat and Shehab El-Din, 1993**).

Diallel crosses have been widely used in genetic research to investigate the inheritance of important traits among a set of genotypes. Analysis of diallel data is usually conducted according to the methods of **Griffing (1956)**, which partition the total variation of diallel data into GCA of the parents and SCA of the crosses according to **Quimio and Zapata (1990), ABE and Futsuhara (1991),Barakat and Shehab El-Din (1993), Torres and Geraldi (2007) and Emara*et al*., (2013).**

Biotechnology offer several valuable techniques such as cell, tissue and organ culture which develop the breeding methods to improve the genetic characters including salt tolerance in the economical crops. Tissue culture generates a wide range of genetic variation in plant species, which can be incorporated in plant breeding programs. By *in vitro* selection, mutants with useful agronomic traits, i.e., salt or drought tolerance or disease resistance can be isolated in a short

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duration. However, the successful use of somaclonal variation is very much dependent on its genetic stability in the subsequent generations (**Mercado *et al*., 2000, Jain, 2001, El-Aref, 2002**).

The main objectives of the present study were to (i) estimate the general performance of the parental lines and their hybrids under salt stress, (ii) estimate GCA and SCA effects as well as heterosis for some *in vitro* traits under salt stress and (iii) identify the best wheat genotype which can be used in wheat breeding programs for salt stress.

MATERIALS AND METHODS

Plant material

Six Egyptian bread wheat (*Triticum aestivum* L.) genotypes namely Gemmiza 9, Gemmiza 1, Giza 164, Giza 163, Giza 160 and Giza 157 were grown to establish the experimental materials for this study. These materials were provided from Agriculture Research Center (ARC), Giza, Egypt. Details of the seven cultivars studied are presented in **Table 1.**

Table 1: Names of the six Egyptian wheat genotypes, their pedigree and year of release.

Genotypes	Pedigree	Year of release
Gemmiza 9	**Ald‘‘s’’/Huac‘‘\s’’//CMH74A.630/5x CGM.4583-5GM-1GM-0GM**	**2000**
Gemmiza 1	**Maya 74/On//1160.147/3/Bb/Gall/4/Chat‘‘s’’ CM58924-1GM-OGM**	**1991**
Giza 164	**Kvz/Buha ‘‘s’’//Kal/Bb CM33027-F-15 M-500y-0 M**	**1987**
Giza 163	***T. aestivum*/Bon//Cno/7C CM33009-F-15 M-4Y-2 M-1 M-1 M-1Y-0 M**	**1987**
Giza 160	**Chenab70/Giza 155**	**1982**
Giza 157	**Giza 155//Pit 62/LR 64/3/Tzpp/Knott**	**1977**

The grains of the six parents and their 15 F_1 crosses were sown in 2012/2013 at experimental farm of Faculty of Agriculture, Menoufia University, Egypt. This study was carried out in Botany Department, Faculty of Science, Menoufia University, Egypt.

Tissue culture conditions

Callus cultures for the six genotypes and their hybrids (F_1) were induced from mature embryos following procedures outlined by **Ozias-Aktins and Vasil (1983)**. Wheat spikes were harvested when the grain was matured. The grains were soaked in sterile water for 24hours, then were rinsed in 70% ethanol for 30seconds, sterilized in Clorox (<5% sodium hypochlorite) for 15minutes and washed with sterile distilled water for several times. After that mature embryos

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were excised and cultured with the scutellum in contact with medium. The culture medium contained the inorganic components of Murashige and Skoog medium (MS) (1962), plus 2.5 mg/L 2,4-dichlorophenoxyacetic acid (2, 4-D), 3%sucrose, 150 mg/L L-asparagine, 160 mg/l thiamine- HCl and 0.8% agar were used in ten replicates (Tissue culture tubes). The medium was adjusted to pH 5.8 and autoclaved for 15 minutes at 121°C and the cultures were incubated at 26°C. Callus was sub cultured at 3weeks intervals until enough callus weight was obtained to start growing on the stress media.

Salt stress

Salt stress were carried out on callus of the six genotypes and their hybrids F_1 using the same medium supplemented with two different concentrations of NaCl (4000and 12000 ppm). Ten tissue culture tubes of each genotype and their hybrids were used for each NaCl level. The fresh weight of callus grown on NaCl containing media was determined by weighting callus before and after three weeks **(Abdel-Hady, 2006)**. Relative growth rate (RGR) = $[W_2-W_1]/GP$ was estimated according to **(Birsin and Ozgen, 2004),** where W_1 and W_2 are the initial and final weight of callus and GP is the growth period, respectively. The time interval between two consecutive measurements was twenty-one days. Callus growth index (CGI) or increasing value of callus fresh weight was calculated as: $CGI = (W_1-W_0)/W_0$ **(Abdelsamad *et al*., 2007)**: where, W_0 is the weight of callus before treatment and W_1 the final weight of callus after three weeks of treatment. Callus growth index was calculated for three levels of salt stress. *In vitro* salt tolerance index (IN-STI): IN-STI was calculated according to the formula of **Al-Khayri and Al-Bahrany (2004)**: IN-STI= RGR treatment/ RGR control where, RGR = relative growth rate and was measured by the formula of **Birsin and Ozgen (2004).**

Statistical analysis

Better-parent heterosis (BPH) for each trait of individual cross was expressed as the percentage increase of F_1 performance above the better-parent (BP) performance (Mather, 1949).The general (GCA) and specific combining ability (SCA) analysis were computed according to **Griffing (1956)** designated as Method 2, Model 1. The data were used to predict heterotic effects of F_1 hybrids.

RESULTS AND DISCUSSION

The genotypes mean performances for the *in vitro* studied traits are given in **Table 2**. Genotypes, parents and the resultant fifteen hybrid combination mean square were found to be highly significant for most *in vitro* traits studied under all different salt stress (0, 4000, 12000 ppm), which indicate overall differences among these populations for all traits **Table 3**. Parent vs.

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crosses mean square estimates, as an indication to average heterosis overall crosses were found to be highly significant for most *in vitro* studied traits.

I. Heterosis

Useful heterosis, expressed as the percentage deviations of the 15 F_1 hybrids mean performance over their respective better–parents (desirable) for each studied traits are presented in **Table 4**. The heterosis effects were observed in all studied traits at the three levels but the degree of heterosis showed variations from trait to trait. High positive values of heterosis would be of interest in most traits under investigation and would be useful for the wheat breeder's point of view. For callus weight (CW_1), seven hybrids showed highly significant positive desirable heterosis under control the three stress levels (L_1, L_2 and L_3) which ranged from 0.24% to 187.29% for the hybrids Gemmiza 1 x Giza 157 and Giza 163 x Giza 157, respectively. As for callus weight (CW_2), four hybrids showed highly significant positive desirable heterosis under control the three stress levels (L_0, L_1 and L_3) which ranged from 5.87% to 155.55% for the hybrids Giza 164 x Giza 160 and Giza 164 x Giza 157, respectively. Concerning callus relative growth rate, one hybrids showed highly significant positive desirable heterosis under control the three stress levels (L_0, L_1 and L_3) for the hybrid Gemmiza 9 x Giza 160. As for callus growth index under control, one hybrid showed highly significant positive desirable heterosis under control the three stress levels (L_1, L_2 and L_3) for the hybrid Gemmiza 9 x Giza 160.Similar results were also previously obtained by **Abdel-Hady (2006), Nazan (2008), Afzal *et al.* (2010), Elyasi *et al.*(2012), Khaled *et al.* (2013) and Islam *et al.*(2015)**.

II. Combining ability

Both general and specific (GCA and SCA) combining ability variances were found to be significant for most *in vitro* studied traits **Table 3**. This indicates the importance of both additive and non-additive genetic variances in determining the performance of these characters. The GCA/SCA ratios were found more than unity for most *in vitro* studied traits, indicating that additive gene action had a greater importance in the inheritance for these *in vitro* traits. For *in vitro* traits similar results were obtained by **Salem (2009), Akbar *et al.* (2010), Seleem and Koumber (2011), Akram *et al.* (2011), Brahim and Mohamed (2014) and Kalharo *et al.* (2015).**

II. a. General combining ability (gi)

Estimates of the GCA effects (gi)of the parental lines for all *in vitro* traits are presented in **Table 5**. High positive GCA effects would be of interest in most traits under investigation and would be useful for the wheat breeder's. Concerning callus weight (CW_1), one wheat genotype **Giza 157**

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showed highly significant positive GCA effect under the three stress levels (L_0, L_1 and L_3), revealing that this wheat genotype could be considered as good combiner for this trait under salt stress conditions**.** With regard to callus weight (CW_2), one wheat genotype **Giza 157** showed highly significant positive GCA effect under the three stress levels (L_0, L_1 and L_3), revealing that this wheat genotype could be considered as good combiner for this trait under salt stress conditions**.** As for callus growth rate, no significant positive GCA effect under the three stress levels (L_0, L_1 and L_3) was detected**.** The only one wheat genotype, Gemmiza 1, exhibited highly significant positive estimates of GCA effect for callus growth index, proving to be excellent combiner for this trait. It could be concluded that the two Egyptian wheat genotype, Gemmiza 1 and Giza 157, which proved to be excellent combiners for most *in vitro* traits under the three stress levels (L_0, L_1 and L_3), would be of practical interest in a breeding program toward developing high salt tolerance genotypes because of their superiority in, at least, two of the studied traits. The results are in accordance for tissue culture traits with those of **Abdel-Hady (2006) and Khaled *et al.*(2013).**

II. b. Specific combining ability ($\hat{s}_{ij}$)

Estimates of the specific combining ability effects ($\hat{s}_{ij}$) for the parental combinations for all studied traits are given in **Table 6.** For most studied traits, the hybridGemmiza9 x Gemmiza1 of the fifteen hybrid combinations was detected to exhibit highly significant desirable SCA effects.

II. Specific combining ability (SCA)

Estimates of the specific combining ability effects (si) of the parental combinations for *in vitro* trait are given in **Table 6**. Three of fifteen hybrid combinations studied showed highly significant positive SCA effects for callus weight (CW_1), two wheat genotypes, Giza 163 and Giza 157, were found to be excellent combiners, therefore, the three hybrid combinations Gemmiza 9 x Gemmiza 1, Giza 164 x Giza 163 and Giza 164 x Giza 157 could be of practical importance in a breeding program for developing either hybrid wheat or pure lines. Since it had significant SCA effect for trait in view and contained a good combiners **Table5**. Regarding to callus weight (CW_2), two hybrid showed significant positive SCA effects. The two hybrid combination, Gemmiza 9 x Gemmiza 1 and Giza 164 x Giza 157, showed significant positive useful heterosis**Table5**. Also, the only one wheat genotype, Giza 157proved to be a good combiners for callus weight (CW_2). These crosses could be of practical importance in a breeding program. Since it had significant SCA effect for the trait in view and contained a good combiners. For callus growth rate, no hybrids showed highly significant positive SCA effects. The results obtained here, concerning GCA and SCA effects, could indicate that the excellent hybrid combinations, which showed desirable SCA effects, were obtained from crossing (good x good), (good x poor) and (poor x poor) combiners. Consequently, it could be concluded that

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GCA effects of the parental lines were, generally, unrelated to the specific combining ability effects of their respective crosses. This conclusion, also, was drawn by **Salem (2009), Akbar *et al.* (2010), Seleem and Koumber (2011), Akram *et al.* (2011), Brahim and Mohamed (2014) and Kalharo *et al.* (2015).**

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Table 2: The genotypes mean performances for *in vitro* traits under three salt stress level.

Genotypes	Callus weight 42 days (gm)			Callus weight 63 days			Callus Relative growth index			Callus growth index			*In vitro* tolerance	
	L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	L_1	L_2
P1	0.11*	0.19**	0.20**	0.18*	0.22**	0.25**	0.00	0.0015	0.00	1.07*	0.19	0.21	0.56	0.81
P2	0.03	0.10 **	0.11*	0.08	0.19*	0.16**	0.00	0.0044	0.00	2.80**	1.35	0.40	2.81**	1.29
P3	0.04	0.10 **	0.11*	0.11	0.22**	0.16**	0.00	0.0058	0.00	1.54**	1.43	0.87	2.01**	0.94
P4	0.10*	0.12 **	0.10*	0.23**	0.20*	0.15**	0.01*	0.0038	0.00	1.66**	0.74	0.51	1.03	0.64
P5	0.09*	0.18 **	0.21**	0.15*	0.22**	0.26**	0.00	0.0018	0.00	0.65	0.16	0.25	2.01**	1.47
P6	0.10*	0.11 **	0.10*	0.17*	0.39**	0.16**	0.00	0.0134	0.00	0.83	3.75**	0.70	4.55**	0.84
P1 x P2	0.24**	0.20 **	0.26**	0.30**	0.24**	0.39**	0.00	0.00	0.01**	0.25	0.25	0.49	1.46	3.81**
P1 x P3	0.17**	0.11 **	0.17**	0.21**	0.17*	0.24**	0.00	0.00	0.00	0.27	0.52	0.51	1.24	2.01*
P1 x P4	0.09*	0.09 *	0.11*	0.19*	0.18*	0.16**	0.00	0.00	0.00	1.13*	1.10	0.50	0.95	0.55
P1 x P5	0.05	0.08 *	0.08*	0.13	0.17*	0.14**	0.00	0.00	0.00	1.42*	1.18	0.77	1.45	0.81
P1 x P6	0.27**	0.12 **	0.25**	0.48**	0.14	0.35**	0.01*	0.00	0.00	0.83	0.19	0.40	0.14	0.56
P2 x P3	0.05	0.08 *	0.09*	0.10	0.15*	0.16**	0.00	0.00	0.00	1.11*	0.87	0.76	1.47*	1.49
P2 x P4	0.13**	0.08 *	0.10*	0.17*	0.17*	0.15**	0.00	0.00	0.00	0.30	1.07	1.77**	2.72**	1.48
P2 x P5	0.13**	0.03	0.10*	0.15*	0.14	0.17**	0.00	0.01	0.00	0.24	5.16**	0.66	5.36**	3.02**
P2 x P6	0.10*	0.15 **	0.17**	0.12	0.26**	0.25**	0.00	0.01	0.00	0.29	0.63	0.82	5.25**	4.41**
P3 x P4	0.23**	0.23 **	0.23**	0.30**	0.33**	0.29**	0.00	0.00	0.00	0.35	0.46	0.27	1.26	0.83
P3 x P5	0.15**	0.21 **	0.24**	0.36**	0.29**	0.27**	0.01*	0.00	0.00	1.43**	0.45	0.13	0.54	0.17
P3 x P6	0.25**	0.22 **	0.28**	0.45**	0.49**	0.37**	0.01*	0.01	0.00	0.85	1.19	0.33	1.41	0.48
P4 x P5	0.27**	0.27 **	0.16**	0.32**	0.35**	0.25**	0.00	0.00	0.00	0.19	0.29	0.57	2.47**	2.95**
P4 x P6	0.30**	0.28 **	0.19**	0.50**	0.37**	0.22**	0.01*	0.00	0.00	0.68	0.33	0.21	0.59	0.18
P5 x P6	0.16**	0.26 **	0.35**	0.28**	0.33**	0.40**	0.01*	0.00	0.00	0.74	0.29	0.13	0.65	0.37
L.S.D. at 0.05	0.09	0.07	0.08	0.14	0.15	0.10	0.00	0.01	0.00	1.01	1.97	0.89	1.47	1.92
L.S.D. at 0.01	0.12	0.09	0.11	0.19	0.20	0.13	0.01	0.01	0.00	1.34	2.63	1.19	1.96	2.56

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***and ** significant at *the P < 0 .05* and the *P < 0.01* levels of probability, respectively.**

L_1= 0 Nacl (Control) L_2= 4000 ppm NaCl L_3= 12000 ppm NaCl

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Table 3: Mean square estimating of ordinary and combining ability analysis for *in vitro* traits under salt stress.

S.O.V.	df	Callus weight (42)days (gm)			Callus weight (63) days			Callus relative growth index			Callus growth index			*In vitro* tolerance	
		L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	L_1	L_2
Replicates	2	0.02	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.13	1.48	1.73	0.54	5.40
Genotypes	20	0.02**	0.02**	0.02**	0.05**	0.03**	0.02**	0.00**	0.00	0.00	1.23**	4.55**	0.39	6.82**	4.32**
Parents	5	0.00	0.01	0.01	0.01	0.02	0.01	0.00	0.00*	0.00	1.83**	5.33*	0.20	6.01**	0.30
Crosses	14	0.02**	0.02**	0.02**	0.05**	0.03**	0.02**	0.00**	0.00	0.00*	0.59	4.49*	0.49	7.47**	5.79**
Parent. vs Crosses	1	0.11**	0.01*	0.03**	0.17**	0.00	0.06**	0.00	0.00	0.00	7.31**	1.47	0.05	1.72	3.82
GCA	5	0.02**	0.01**	0.01**	0.06**	0.05**	0.02*	0.00**	0.00*	0.00	0.42	3.06	0.33	11.99**	5.15*
SCA	15	0.02**	0.02**	0.02**	0.04**	0.02	0.02**	0.00**	0.00	0.00*	1.51**	5.05*	0.42	5.09**	4.04*
Error	40	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.49	1.86	0.38	1.04	1.77
GCA/SCA	-	0.72	0.81	0.81	1.40	3.04	0.70	1.57	3.31	0.78	0.28	0.61	1.27	2.35	1.27

***and ** significant at *the P < 0 .05* and the *P < 0.01* levels of probability, respectively.**

L_1= 0 Nacl (Control) L_2= 4000 ppm NaCl L_3= 12000 ppm NaCl

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Table 4: percentages of heterosis over batter-parents for *in vitro* traits under three salt stress levels

	Callus weight 42 days (gm)			Callus weight 63 days (gm)			Callus relative growth index			Callus growth index			*In vitro* tolerance	
Genotypes	L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	L_1	L_2
P1 x P2	118.91**	3.82**	30.33**	65.85**	9.02**	60.57**	-21.95**	-52.02**	200.77**	-89.69**	33.77**	22.55**	-48.09**	196.61**
P1 x P3	52.17**	-40.71**	-17.40**	17.68**	-24.99**	-3.06**	-39.35**	-55.50**	28.92**	-82.34**	179.34**	-41.59**	-38.49**	114.75**
P1 x P4	-14.86**	-52.38**	-45.37**	-16.37**	-17.46**	-33.37**	-23.16**	15.60**	15.84**	-31.93**	485.98**	-0.70	-7.54**	-32.31**
P1 x P5	-53.49**	-58.26**	-59.26**	-29.37**	-23.10**	-46.91**	10.65**	147.83**	4.23**	32.88**	532.46**	203.22**	-27.81**	-45.36**
P1 x P6	144.23**	-36.02**	25.60**	167.96**	-62.83**	41.18**	161.45**	-27.77**	52.58**	-22.00**	-95.02**	-43.27**	-97.01**	-32.52**
P2 x P3	11.44**	-15.62**	-17.82**	-5.62**	-30.77**	-3.09**	-16.97**	-45.27**	18.69**	-54.92**	-39.54**	-13.14**	-47.86**	16.26**
P2 x P4	29.56**	-28.92**	-7.22**	-28.51**	-13.70**	-2.84**	-74.94**	-6.33**	2.69**	-87.82**	-20.62**	248.22**	-3.20**	15.05**
P2 x P5	41.12**	-84.26**	-49.66**	-2.17**	-35.16**	-34.35**	-65.01**	22.92**	29.01**	-90.44**	30.10**	160.25**	90.57**	104.71**
P2 x P6	0.24**	38.53**	49.70**	-32.55**	-32.90**	51.91**	-72.29**	-60.16**	33.50**	-88.44**	-83.08**	16.91**	15.25**	243.04**
P3 x P4	119.03**	93.77**	112.02**	30.74**	49.08**	76.29**	-40.10**	-20.28**	6.35**	-78.98**	-67.56**	-69.09**	-37.17**	-10.94**
P3 x P5	70.06**	11.28**	16.37**	136.74**	30.83**	5.87**	218.66**	-30.32**	-43.80**	-7.19**	175.79**	-85.12**	-72.99**	-88.36**
P3 x P6	157.43**	103.40**	159.80**	155.55**	24.74**	125.00**	153.27**	-5.28**	47.48**	-44.99**	-68.40**	-62.05**	-68.98**	-49.24**
P4 x P5	167.16**	46.13**	-21.72**	38.09**	57.54**	-1.10**	-65.52**	-1.33**	84.28**	-88.59**	77.90**	11.75**	23.08**	100.35**
P4 x P6	187.29**	139.21**	82.14**	114.51**	-4.21**	36.09**	56.08**	-67.88**	-42.38**	-59.20**	-91.09**	-70.07**	-87.12**	-78.21**
P5 x P6	70.97**	39.25**	71.72**	62.24**	-14.47**	55.90**	51.67**	-72.92**	-26.21**	-11.11**	-92.25**	-81.71**	-85.73**	-74.93**
L.S.D. at 0.05	0.09	0.07	0.08	0.14	0.15	0.10	0.00	0.01	0.00	1.01	1.94	0.89	1.47	1.92
L.S.D. at 0.01	0.12	0.09	0.11	0.19	0.20	0.13	0.01	0.01	0.00	1.43	2.59	1.19	1.96	2.56

***and ** significant at *the P < 0 .05* and the *P < 0.01* levels of probability, respectively.**

L_1= 0 NaCl (Control) L_2= 4000 ppm NaCl L_3= 12000 ppm NaCl

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Table 5: Estimating of general combining ability effect for the parental genotypes for *in vitro* traits under salt stress.

	Callus weight 42 days (gm)			Callus weight 63 days (gm)			Callus relative growth index			Callus growth index			*In vitro* tolerance	
Genotypes	L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	L1	L2
P1	0.01	-0.03*	0.03	0.00	-0.15**	0.05*	0.00	-0.0055	0.00	-0.064	-1.35**	-0.25	-2.606**	-0.13
P2	-0.12**	-0.12**	-0.09**	-0.25**	-0.15**	-0.08**	-0.01**	-0.0011	0.00	0.597*	1.31**	0.58**	3.217**	2.65**
P3	-0.04*	-0.01	0.01	-0.01	0.04	0.00	0.00	0.0024	0.00	0.330	-0.32	0.00	-1.266**	-1.06*
P4	0.08**	0.05**	-0.08**	0.11**	0.02	-0.10**	0.00	-0.0012	0.00	-0.087	-0.93*	0.21	-1.220**	-0.91*
P5	-0.03	0.05**	0.05*	-0.04	-0.01	0.03	0.00	-0.0028	0.00	-0.337	0.19	-0.37	0.442	0.21
P6	0.09**	0.07**	0.09**	0.19**	0.24**	0.10**	0.00	0.0082	0.00	-0.438	1.10*	-0.17	1.434**	-0.76
L.S.D. at 0.05	0.04	0.03	0.04	0.06	0.07	0.04	0.00	0.00	0.00	0.46	0.90	0.40	0.67	0.87
L.S.D. at 0.01	0.06	0.04	0.05	0.09	0.09	0.06	0.00	0.00	0.00	0.61	1.20	0.54	0.89	1.16

***and ** significant at *the $P < 0.05$* and the *$P < 0.01$* levels of probability, respectively.**

L_1= 0 NaCl (Control) L_2= 4000 ppm NaCl L_3= 12000 ppm NaCl

Table 6: Estimating of specific combining ability effect for the crosses for *in vitro* traits under salt stress.

	Callus weight 42 days (gm)			Callus weight 63 days (gm)			Callus relative growth index			Callus growth index			*In vitro* tolerance	
Genotypes	L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	L_0	L_1	L_2	-2.081*	L2
P1 x P2	0.39**	0.28**	0.32**	0.41**	0.25**	0.49**	0.00	-0.0012	0.01*	-2.498**	-2.38	-0.51	1.726	4.65**
P1 x P3	0.08	-0.09*	-0.06	-0.09	-0.16*	-0.06	-0.01*	-0.0034	0.00	-2.180**	0.07	0.13	0.834	2.97**
P1 x P4	-0.25**	-0.21**	-0.15**	-0.26**	-0.09	-0.18**	0.00	0.0058	0.00	0.817	2.39*	-0.11	0.659	-1.59
P1 x P5	-0.28**	-0.25**	-0.36**	-0.31**	-0.10	-0.40**	0.00	0.0072	0.00	1.921**	1.54	1.27*	-4.273**	-1.93
P1 x P6	0.26**	-0.14**	0.11*	0.51**	-0.42**	0.17**	0.01*	-0.0136	0.00	0.267	-2.36	-0.04	-3.402**	-1.68
P2 x P3	-0.15*	-0.09*	-0.16**	-0.16	-0.21**	-0.17**	0.00	-0.0059	0.00	-0.315	-1.56	0.05	0.323	-1.37
P2 x P4	-0.01	-0.14**	-0.04	-0.09	-0.13	-0.09	0.00	0.0007	0.00	-2.336**	-0.33	2.85**	6.579**	-1.57
P2 x P5	0.08	-0.31**	-0.18**	0.01	-0.19*	-0.17**	0.00	0.0061	0.00	-2.280**	10.82**	0.12	5.238**	1.92
P2 x P6	-0.13*	0.03	-0.02	-0.32**	-0.07	0.00	-0.01*	-0.0051	0.00	-2.032**	-3.67**	0.41	0.419	7.07**
P3 x P4	0.18**	0.18**	0.23**	0.08	0.15	0.24**	0.00	-0.0015	0.00	-1.924**	-0.53	-1.05	-3.402**	0.21
P3 x P5	0.07	0.10*	0.13*	0.40**	0.07	0.05	0.02**	-0.0017	0.00	1.563*	-1.68	-0.89	-1.785*	-2.90*
P3 x P6	0.23**	0.13**	0.21**	0.43**	0.41**	0.28**	0.01*	0.0134	0.00	-0.081	-0.39	-0.48	2.338*	-1.01
P4 x P5	0.32**	0.24**	-0.02	0.17*	0.27**	0.10	-0.01*	0.0012	0.01**	-1.736**	-1.55	0.21	-4.308**	5.29**
P4 x P6	0.27**	0.27**	0.03	0.46**	0.10	-0.05	0.01*	-0.0082	0.00	-0.169	-2.34	-1.06	-5.780**	-2.05
P5 x P6	-0.02	0.18**	0.38**	-0.03	0.00	0.33**	0.00	-0.0086	0.00	0.259	-3.58**	-0.72	1.78	-2.61*
L.S.D. at 0.05	0.11	0.09	0.10	0.17	0.18	0.12	0.00	0.01	0.00	1.22	2.39	1.08	2.37	2.32
L.S.D. at 0.01	0.15	0.11	0.13	0.23	0.24	0.16	1.42	0.01	0.00	19.56	3.18	1.44	-2.081*	3.10

***and ** significant at *the P < 0 .05* and the *P < 0.01* levels of probability, respectively.**

L_1= 0 NaCl (Control) L_2= 4000 ppm NaCl L_3= 12000 ppm NaCl

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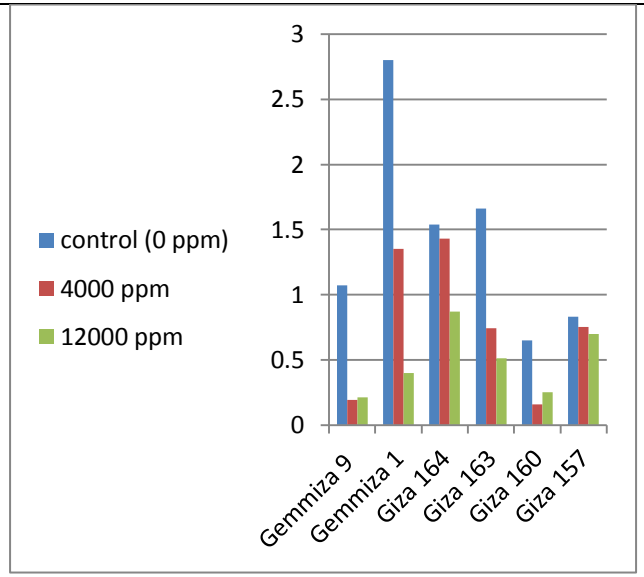
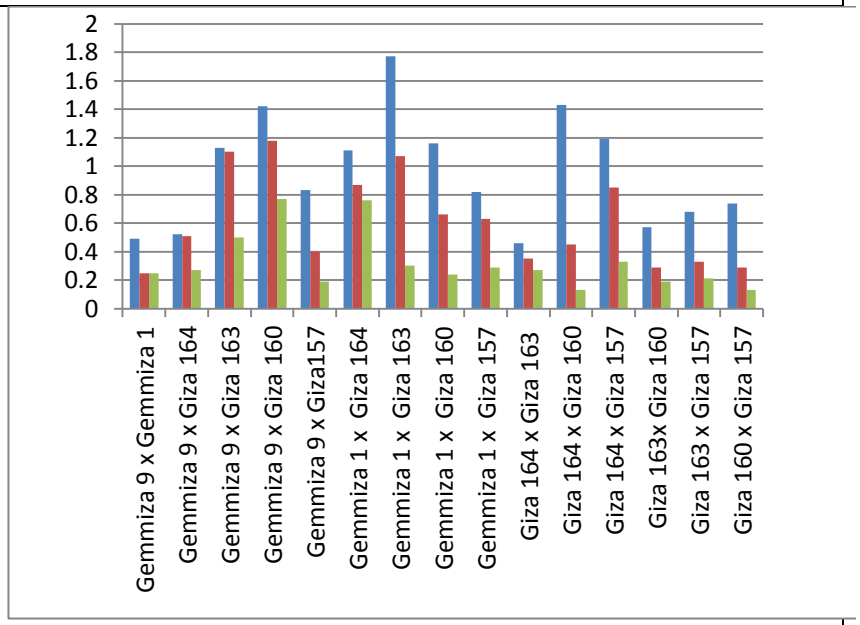
	Parents	Hybrids
Callus Growth Index		

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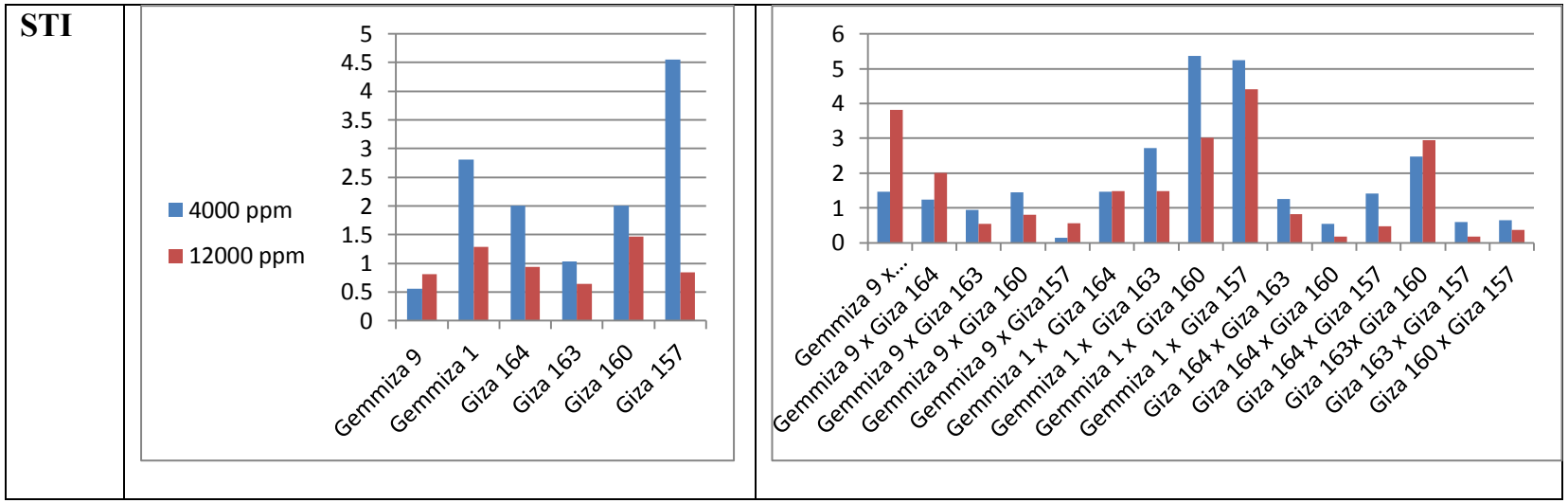

Fig.1: Mean performance for all genotypes for both callus growth index and STI traits

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REFERENCES

Abdel-Hady, M.S. (1999). Wheat plantlets production *via* shoot tips under salinity stress. J. Agric. Sci. Mansoura Univ. 24(7): 4841-4857.

Abdel-Hady, M.S.S. (2006). Heterosis and Combining Ability Effects for Callus Growth of Wheat (*Triticum durum*Desf.) *in vitro*. J. Appl. Sci. Res., 2(6): 360-363.

Abdelsamad, A.; El-Sayed, O.E. and Ibrahim, F. (2007). Development of drought tolerance haploid wheat using biochemical genetic markers on in vitro culture. J. Appl. Sci. Res., 3(11): 1589-1599.

ABE, T. and Futsuhara, Y.(1991). Diallel analysis of callus growth and plantregeneration in rice seed callus.*The Japanese J. Genet.,* 66: 129-140.

Afzal, A.; Rashid, H.; Khan, M. H.; Chaudhry, Z. and Malik, S.A. (2010). High frequency regeneration system optimization for wheat cultivar INQILAB-91.*Pak. J. Bot*., 42(3): 1857-1862.

Akbar, M.; Anwar, J.; Hussain, M.; Iqbal, M.M. and Sabir, W. (2010). Heterosis and heterobeltiosis for grain yield and improvement in bread wheat.*J. Agric. Res.,* 48(1): 15-23.

Akram, Z.; Ajmal, S.U.; Khan, K.S.; Qureshi, R. and Zubair, M. (2011). Combining ability estimates of some yield and quality related traits in spring wheat (*Triticum aestivum* L.). *Pak. J. Bot.*, 43(1): 221-231.

Al-Khayri, J.M. and Al-Bahrany, A.(2004). Growth, water content and proline accumulation in drought stressed callus of date palm. Biol. Plant, 48(1): 105-108.

Ashraf, M. (1994). Breeding for salinity tolerance in plant. Crit. Rev. Plant Sci, 13: 17-42.

Barakat, M. N. and Shehab El-Din, T.M. (1993). An in vivo and in vitro analysis of a diallelcross in wheat (*Triticumaestivum* L.). J. Genet. & Breed., 47: 211-216.

Birsin, M.A. and Ozgen, M.(2004).A comparison of callus induction and plant regeneration from different embryo explants of triticale (X TriticosecaleWittmack). Cellular Mol. Biol. Let., 9: 353-361.

Brahim, B. and Mohamed, B. (2014). Analysis of diallel crosses between six varieties of durum wheat in semi-arid area. *Afri. J. Biotech.,* 13(2): 286-293.

Diaz De Leon, J.L.; Escoppinichi, R.; Geraldo, N.; Castellanos, T.; Mujeeb-Kazi, A. and Roder, M.S. (2011). Quantitative trait loci associated with salinitytolerance in field grown bread wheat. Euph.,181:371-383.

Diaz De Leon J.L.;Escoppinichi R.; Zavala-Fonseca R. and Mujeeb-Kazi A. (2000).A sea-water based salinity testing protocol and the performance of a tester set of accumulated wheat germplasm. Ann. Wheat Newsletter., 46: 88-90

ISSN: 2455-6939

Volume:03, Issue:01

El-Aref, H.M. (2002). Employment of maize immature embryo culture for improving drought tolerance.[3]rd Scientific Conference of Agriculture Sciences, Fac. Agric., Assiut Univ., Assiut, Egypt, 20-22 Oct., 463-477.

El-Sayed, A.M.S.; El-Naggar, H.M.H.; Abbas, M.S and Gaber, E.I. (2016). Genetic Selection for Salt Tolerance in Some Egyptian Wheat Genotypes (*Triticum aestivum* L.) *via* Tissue Culture. Plant Tissue Cult.& Biotech., 26(1): 25-36.

Elyasi, P.; Farshadfar, E. and Aghaee, M. (2012). Response of Bread Wheat Genotypes to Immature Embryo Culture, Callus Induction and Drought Stress.*Curr. Res. J. Biol. Sci.,* 4(4): 372-380.

Emara, H; Salem, K.; Abdel-halim, A. and Abdel-kowy, R. (2013) . An in vivo and *in vitro* traits analysis of a diallelcrossin rice (*Oryza sativa* L.). 8th Egyptian Plant Society of breeding Conference, 6-7 May 2013,, At Faculty of Agriculture, Kafer El-Sheik, Egypt.

Flowers, T.J.; Garcia, A.; Koyama, M. and Yeo, A.R.(1997). Breeding for salt tolerance in crop plants-therole of molecular biology. Acta Physiol. Plant., 19:427-433.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel system. Aust. J. Bio. Sci., 9: 463-493.

Hala, A.A.; Abdel Gaffar, E.S. andMutasim, M.K. (2012). Establishment of an efficient callusinduction and plant regeneration system in some wheat (*Triticumaestivum*L.) cultivars grown in Sudan. African J. Biotech.Febr., **11**(16): 3793-3799.

Hollington, P.A. (1998). Technological breakthroughs in screening and breeding wheat varieties for salt tolerance. Proceedings of the National Conference on Salinity Management in Agriculture CSSPI, Karnal, India, 273-289.

Islam, M.M.; Remme, R.N.; Biswas, D. and Yesmin, F. (2015). In vitroplant regeneration from mature embryos of wheat (*Triticumaestivum* L.).Int. J. Multidiscip. Res. Develop., 2(4): 669-672.

Jain, S.M. (2001). Tissue culture-derived variation in crop improvement.Euph., 118:153–166.

Kalhoro, F.A.; Rajpar, A.A.; Kalhoro, S.A.; Mahar, A.; Ali, A.; Otho, S.A.; Soomro, R.N.; Ali, F. and Baloch, Z.A. (2015). Heterosis and combining ability in F_1 population of hexaploid wheat (*Triticumaestivum* L.). *Amer. J. Pl. Sci.,* (6):1011-1026.

Khaled, A.G.A.; Hamam, K.A.; Motawea, M.H. and El-Sherbeny, G.A.R. (2013). Genetic studies on tissue culture response and some agronomical traits in Egyptian bread wheat. *J. Gene. Eng. Biotech.,* 11(2): 79–86.

Ma, L.; Zhou, E.;Huo, N.; Zhou, R.; Wang, G. and Jia, J.(2007). Genetic analysis of salt tolerance in a recombinant inbred population of wheat (*Triticumaestivum* L.). Euph., 153:109-117.

Mercado, J.A.; Sancho, C.; Jimenez, B.; Peran, U.; Pliego, A. and Quesada, M. (2000). Assessment of *in vitro*growth of apical stem sections and adventitious

organogenesis to evaluate salinity tolerance in cultivated tomato.Plant Cell, Tissue and Organ Culture62: 101-106.

Mona A Ismail (2014). Exogenous proline induced changes in SDS-PAGE Protein profile for salt tolerance in wheat (*Triticumaestivum* L.) seedlings. Research J Pharmaceutical, Biological and Chem. Sci., 5(4): 748.

Nazan, D. (2008). Genetic analysis of grain yield per spike and some agronomic traits in diallel crosses of bread wheat (*Triticumaestivum* L.). *Turk. J. Agric.,* 32: 249-258.

Ozias-Aktins, P. and **Vasil J.K.** (1983). Callus induction and growth from the mature embryoof*Triticumaestivum*(wheat). Protoplasma, 115, 104.

Peng, J. and Hodges, T.K. (1989). Genetic analysis of plant regeneration in rice (*Oryzasativa* L.). In vitro Cell and Dev. Biology., 25: 91-94.

Qu, Y.; Mok, M.C.;Mok, D.W.S. and Stang, J.R. (1988). Phenotypic and cytological variation among plants derived from anther cultures of *Liliumlongiflorum*. *In vitro* Cellular, Developmental Biology, 24:471-476.

Quimio, C.A. and Zapata, F.J. (1990). Diallel analysis of callus induction and greenplantregeneration in rice anther culture. Crop Sci., 30:188–192.

Salem, K.F.M. (2009). Relationship between genetic diversity based on SSRs markers with heterosis and combining ability in diallel cross of bread wheat (*Triticumaestivum* L.). Minufiya J. Agric. Res.,34 (6): 2159-2178.

Salem, K. and Mattar, M. (2014). Identification of microsatellite alleles for salt tolerance at seedling stage in wheat (*Triticumaestivum* L.).Life Science Journal, 11(12s): 1064-1073.

Salama, E.A.;Abido, A.;Khaled, A.E. andAbdelsalam, N.R. (2013). Embryo callus inductionand regeneration of some Egyptian wheat cultivars. Res. J. Agric. & Biol. Sci., 9(2): 96-103.

Salam, A.G. (2002). Current status of durum wheat in Egypt and future prospects.http://www.Fineprint.com.

Seleem, S.A. and Koumber, R.M.A.(2011). Estimation of combining ability and gene action in the F1 and F2 generations in some breed wheat crosses.Minufiya J. Agric. Res., 36 (6): 1627-1648.

Torres, E.A. and Geraldi, I.O. (2007). Partial diallel analysis of agronomic characters in rice (*Oryza sativa* L.). Genetics and Molecular Biology, 30(3) : 605-613.

Tuna, A.L.; Kaya, C.; Dikilitas, M. and Higgs, D. (2008).The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environ. Exp. Bot., 62: 1-9.

Williams, G.D.V.;Fautley, R.A.; Jones, K.H.; Stewart, R.B. and Wheaton, E.E. (1988). Estimating Effects of Climatic Change on Agriculture in Saskatchewan, Canada. In M.L.

ISSN: 2455-6939

Volume:03, Issue:01

Parry et al. (ed.) The Impact of Climatic Variations on Agriculture. Vol. 1 Assessment in Cool Temperate and Cold Regions. Reidel Publ. Co. Dordrecht., 219-379.

Yang Y.W.; Newton, R.J. and Miller , F.R. (1990). Salinity tolerance in sorghum. II. Cell culture response to sodium chloride in S. bicolor and S. halepense. Crop Sci., 30:781-785.