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Combining ability of plant regeneration in some bread wheat genotypes under salinity stress conditions

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Abstract

Salt tolerant calli were obtained from anther culture of nine genotypes of bread wheat and their 36 F1 crosses to estimate combining ability effects for plant regeneration and green plantlet regeneration under different salt stress concentrations (control, 4, 8 and 12 g/L NaCl). The analysis of variance showed that salt stress levels mean squares were found to be highly significant for all in vitro studied traits. Considerable genetic variation among studied genotypes was observed for all in vitro studied traits under the four salinity stress treatments except albino plantlet regeneration at the two stress levels of 8 and 12 g/L NaCl. The highest frequencies of plant regeneration and green plantlet regeneration under the four salinity stress treatments were recorded for the two crosses P1 × P3 and P3 × P9, while the lowest ones were obtained in the two varieties; Giza-171 (P4) and Sids-14 (P6). High ratio of GCA/SCA was found more than unity for all in vitro studied traits under the four stress levels indicating that predominant role of additive gene action in the inheritance of all in vitro studied traits. Results showed that the parents Gemmeiza-7 (P1), Gemmeiza-11 (P3) and Line-Azhar-1 (P9) were the best GCA for green plant regeneration under the four salt stress levels. The five crosses (P1 × P3, P1 × P7, P3 × P9, P4 × P8 and P5 × P6) appeared the best SCA effects for plant regeneration and regenerated green plantlet under salinity stress levels.

Keywords: bread wheat, anther culture, combining ability, salinity stress.

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1. Introduction

Bread wheat (*Triticum aestivum* L.) is considered the major cereal crops used for human consumption and serves as a major source of food worldwide and is the most important cereal crop in Egypt however, only 40 % of its annual domestic demand can be produced (Nawara *et al*., 2017; Salam 2002). In 2021/22 the planting area of 3.4 million feddans (1.40 million hectares) this area produces 9.460 million tons, and the consumption is about 16.768 million tons (USDA 2022). Egypt, however still imports about 6.784 million tons of wheat to cover its consumption. This constituted a high level of import, and food security becoming a serious problem. The government strategic plan is to increase the total agricultural land by adding newly reclamation land, due to limitation of cultivated land (Amin *et al*., 2010). Therefore, it is necessary to increase wheat production to realize food security. An important objective of the Egyptian government is consequently to reduce the dependence on imported wheat by enhancing grain yield production and cultivating modern wheat cultivars in new reclaimed soil which suffer from salinity also, clay soil as old soil gains salinity from water irrigated salinity (Kandil *et al*., 2013). Increasing wheat production to reduce the gap between production and consumption is the main target of wheat breeders. Despite many efforts of wheat breeders, yield losses due to a biotic stress such as water stress, salinity (Thanaa and El-Hussin, 2013). Salt stress is one of the most prevalent abiotic stresses in the world that negatively impacts plant growth (Khan, 2011). The saline area is three times larger than the soil used for agriculture (Khan *et al*., 2016). Consequently, salinity has been a serious problem in several parts of the world including Egypt. Salinization of proper agricultural land is suggested to decrease 50 % of the availability of cultivable land by the year 2050 (Minhas *et al*., 2017; Shaimaa *et al*., 2021). Salinity decreases plant growth and yield due to increased effectiveness in water use and changes the plant metabolism (Khedr *et al*., 2022; Rani *et al*., 2019; Sahin *et al*., 2020). About 6% of the total land area in the world and 20% of the irrigated land is suffering from salt stress (Ghonaim *et al*., 2021). Egypt is one the countries suffer from severe salinity problems as 33% of the cultivated land, which comprises only 3% of total land area in Egypt, is already salinized due to low precipitation (less than 25 mM annual rainfall) and irrigation with saline water (Al-Naggar *et al*., 2015; Ghassemi *et al*., 1995; Mattar *et al*., 2009; Shaimaa *et al*., 2021). Wheat is the most important and widely cultivated food cereal crop in Egypt. The most efficient way to increase wheat yield is to improve the salt tolerance of wheat genotypes (Ashraf *et al*., 2005; Gadallah *et al*., 2017; Khan *et al*., 2003; Pervaiz *et al*., 2002; Ragab and Kheir, 2019). It is known that anther culture is one of the efficient biotechnology methods in plant breeding of wheat to produce doubled haploid lines. Anther culture (androgenesis) is to obtain

haploid embryo using immature pollens (microspores) in anthers cultivated on nutrition media. This procedure usually needs a short time to be conducted (only one generation) and could accelerate the production of new varieties with improved traits (Barakat *et al*., 2012). The success of anther culture ability in wheat, as other crops, is found to be influenced by genotypes (Andersen *et al*., 1988) donor plant growth conditions (Broughton 2011; Castillo *et al*., 2015; Coelho *et al*., 2018; Lantos *et al*., 2013; Nielsen *et al*., 2015; Orlowska *et al*., 2020; Sen, 2017; Wang *et al*., 2019), the developmental stage of microspores (Haggag and El-Hennawy 1996), pre-culture treatments, and media components (Lazaridou *et al*., 2005 and Lantos and Pauk 2021). To be successful in a breeding program using anther culture for doubled haploid production, workers should include genotypes with high regeneration ability. Haploids production are of great importance; which are used in achieving homozygosity in quick way, facilitating genetic and could make a significant contribution to breeding wheat varieties (Hassawi *et al*., 2005; El-Hennawy *et al*., 2011; Kaushal *et al*., 2014; Siddique 2015). Several studies dealing with anther cultures agree that the genotypic effect is the main limiting factor of in vitro androgenesis. Many wheat genotypes, as well as F1 and F2 breeding combinations, are incapable of morphogenesis in anther culture, which makes this method too expensive to be used for routine purposes (Yermishina *et al*., 2004). Therefore, one of the proposed approaches was to use anther culture only with responsive genotypes (Andersen *et al*., 1988). On the other hand, the responsiveness of parental genotype to anther culturing affects the responsiveness of hybrid combinations involving them (Al-Ashkar, 2014; Zamani *et al*., 2003). Moreover, there is evidence that F1 hybrids have a higher androgenetic capacity than the parental forms. The genetic research and breeding programs depend on the proper diagnosis of the conditions of quantitative trait inheritance. During the selection process, the information about the combining ability of parental components used for crossbreeding is very important. This knowledge is essential for proper selection of suitable parents in identifying promising hybrids. A common method used in a classical genetic analysis is diallel crossing applied to evaluate the combining ability of parents and progeny generations. One possible way to analyze diallel crosses is the method proposed by Griffing (1956), which divides the total genetic variance for the GCA of parents and the SCA of obtained hybrids. The knowledge of combinatorial abilities helps to understand the nature of the action of genes involved in the expression of quantitative traits and predicts the value of further generations (Machikowa *et al*., 2011). The present investigation was conducted to study anther culture response of some wheat parental genotypes and their F1 crosses under salinity stress treatments as well as to gather information on the genetic

behavior of anther culture response under NaCl stress treatments in a nine – parent half diallel cross of bread wheat.

2. Materials and methods

2.1 Experimental site and treatments description

The present investigation was carried out at the Cell and Tissue Culture Laboratory as well as the Experimental Farm of the Agronomy Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt, during the period from 2019 to 2022. Nine parental genotypes of bread wheat namely, Gemmeiza-7 (P1), Gemmeiza-9 (P2), Gemmeiza-11 (P3), Giza-171 (P4), Sakha-95 (P5), Sids-14 (P6), Misr-3 (P7) and Shandaweel-1(P8) as well as Line-Azhar-1 (P9) from Prof. Dr. M. A. El-Hennawy Agronomy Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, representing a wide range of diversity for several traits were chosen for this study. In 2019/20 season, the parental genotypes were crossed in all possible combinations excluding reciprocals, to obtain a total of 36 crosses. The Nine parents and their 36 F1 crosses were sown at the Experimental Farm in 2020/21 season to obtain the needed anthers. Whole tillers at boot stage were collected when most microspores were at the mid- to late uninucleate stage of development, as assessed by acetocarmine staining of selected squashed anthers. Inter-ligule length on top of the tiller was used as an indicator of this stage. Tillers with spikes at this stage were clipped off at ground level and tagged. Then, they were put in water and maintained for 6-8 days at 4°C in the dark. After cold pretreatment, the spikes inside flag leaves were surface sterilized with 20 % chlorax solution for 7 min. and rinsed 3-4 times in sterile water. Anthers were aseptically dissected out and cultures in jars containing the N6 induction medium of anther culture (Chu, 1978) supplemented with 2 mg/L 2,4-D, 0.5 mg/L kinetin, 90 g/L sucrose, 7 g/L agar and in the same N6 medium. These jars were incubated first for 5-6 weeks in darkness at 28°C. Completely randomized design was applied in this experiment with 45 genotypes and 10 replicates. Each replicate contained 30 anthers from each spike, which were placed in jar. Embryoids/callus induced from the anthers were transferred to jars containing MS regeneration medium (Murashige and Skoog 1962) supplemented with 0.5 mg/L NAA, 0.5 mg/L kinetin, 30 g/L, sucrose, 6 g/L agar and salt stress levels, the effect of salt stress induced by addition of sodium chloride (NaCl) using four salinity levels (control, 4, 8 and 12 g/L NaCl) in the medium. These jars were incubated for 5-6 weeks at 25-27 °C with 16 h light. In regeneration medium, some callus lost their capacity to make plantlets and turned brown, while others differentiated in green or albino plantlets. Green plantlets without rooting were transferred to rooting medium (half strength MS medium free hormone). The number of green and albino plantlets regenerants

were counted. Green plantlets with adequate root formation were transplanted to small pots with mixture of soil, sand and compost, under plastic cover for three weeks in a growth chamber maintained at 18 °C and 16 h light per day. The regenerated plantlets were transferred to the soil in green house under greenhouse conditions for growth and maturation. The data recorded on all the traits (callus induction, plant regeneration, number of green plantlets and number of albino plantlets) were subjected to analysis of variance (Steel and Torrie 1980) to determine the significant differences among genotypes. The diallel analysis was performed according to Griffing's (1956) method 2 and model 1.

3. Results and Discussion

3.1 Anther culture response of wheat parental genotypes and their F1 crosses for callus induction

One of the important factors for haploid production through androgens is the culturing of anthers at the mid to late uninucleate stage. When cultured at this stage there is shifting in the normal pathway of pollen development and after repeated mitotic divisions of the microspores, calli are formed (Haggag and El-Hennawy, 1996). The successful application of anther culture in wheat-breeding programs depend on the good androgenic response of genotypes and the high frequency of plant regeneration. The responses of the anthers of nine wheat parents and their hybrids studied are presented in Table (1). Highly significant differences in response to anther culture were recorded among the nine parental genotypes and their crosses, revealing the presence of genetic diversity in the material used. Callus was obtained from all wheat genotypes. The percentage of anthers that developed calli of parents ranged from 7.33% (Sids-14) to 15.00% (Gemmiza-11). The crosses ranged from 7.00 calli/100 anthers for the cross (P4 × P9) to 24.33 calli /100 anthers for the cross (P3 × P9) (Table 1 and Figure 1). Similar results were found by Tuvesson *et al*., (2000) and Zamani *et al*. (2003).

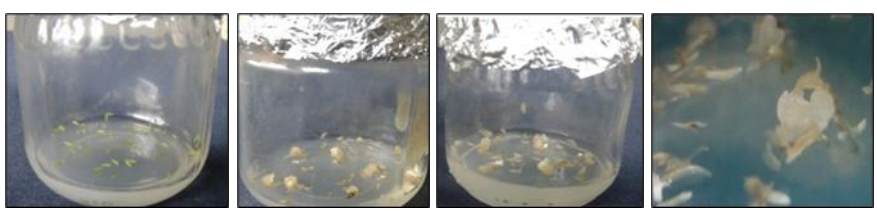

Figure (1): *In vitro* induction of haploid wheat plants through anther culture, formation of callus in the cultured anthers.

Table (1): Anther culture response of F1 hybrids and their respective parents for callus induction.

Genotypes	Number of anthers plated	Number of anthers responded	Callus induction (%)
Gemmeiza –7 (P_1)	300	38	12.67
Gemmeiza – 9 (P_2)	300	33	11.00
Gemmeiza -11 (P_3)	300	45	15.00
Giza -171 (P_4)	300	24	8.00
Sakha - 95 (P_5)	300	28	9.33
Sids -14 (P_6)	300	22	7.33
Misr-3 (P_7)	300	34	11.33
Shandaweel-1 (P_8)	300	36	12.00
Line-Azhar-1 (P_9)	300	43	14.33
$P_1 \times P_2$	300	46	15.33
$P_1 \times P_3$	300	71	23.67
$P_1 \times P_4$	300	26	8.67
$P_1 \times P_5$	300	54	18.00
$P_1 \times P_6$	300	28	9.33
$P_1 \times P_7$	300	60	20.00
$P_1 \times P_8$	300	57	19.00
$P_1 \times P_9$	300	62	20.67
$P_2 \times P_3$	300	32	10.67
$P_2 \times P_4$	300	33	11.00
$P_2 \times P_5$	300	50	16.67
$P_2 \times P_6$	300	24	8.00
$P_2 \times P_7$	300	26	8.67
$P_2 \times P_8$	300	30	10.00
$P_2 \times P_9$	300	28	9.33
$P_3 \times P_4$	300	53	17.67
$P_3 \times P_5$	300	35	11.67
$P_3 \times P_6$	300	41	13.67
$P_3 \times P_7$	300	34	11.33
$P_3 \times P_8$	300	38	12.67
$P_3 \times P_9$	300	75	24.33
$P_4 \times P_5$	300	33	11.00
$P_4 \times P_6$	300	28	9.33
$P_4 \times P_7$	300	55	18.33
$P_4 \times P_8$	300	52	17.33
$P_4 \times P_9$	300	21	7.00
$P_5 \times P_6$	300	53	17.67
$P_5 \times P_7$	300	54	18.00
$P_5 \times P_8$	300	22	7.33
$P_5 \times P_9$	300	27	9.00
$P_6 \times P_7$	300	28	9.33
$P_6 \times P_8$	300	31	10.33
$P_6 \times P_9$	300	35	11.67
$P_7 \times P_8$	300	38	12.67
$P_7 \times P_9$	300	56	18.67
$P_8 \times P_9$	300	31	10.33
L.S.D. 0.05	0.67		
L.S.D. 0.01	0.89		

3.2 Effect of different salt stress treatments on plant regeneration response of wheat genotypes

The analysis of variance for the studied traits under the four salt stress treatments (0, 4, 8 and 12 g/l NaCl) and their combined data is presented in Table (2). Results showed that salt stress levels mean squares were found to be highly significant for all in vitro studied traits, revealing that performance of the tested genotypes differed under the four salt

stress levels. Similar results were also found by Bahman *et al*. (2012) and El-Hennawy *et al*. (2018). Mean squares due to genotypes, parents and crosses were significant or highly significant for all in vitro studied traits under the four salinity stress levels and their combined data except albino plant regeneration under the stress levels of 8 and 12 g/l NaCl. Also, significant or highly significant mean squares due to interaction of genotypes, parents and crosses with NaCl treatments were detected for all in vitro studied traits except albino plant regeneration, indicating that these genotypes varied in their response to NaCl stress levels for all studied traits. These results are in agreement with those obtained by Hentour *et al*. (2020). Parent *vs*. crosses mean squares (Table 2) as an indication to average heterosis overall crosses were found to be significant or highly significant for all in vitro studied traits under the four stress levels and the combined data except green and albino plantlets under the stress level of 12 g/l. Moreover, the interaction of parents vs. crosses with salinity stress levels was found to be significant or highly significant for all *in vitro* studied traits.

Table (2): Mean squares of single (S) and combined (Comb) analysis of variance for the *in vitro* studied traits of wheat genotypes under four salinity levels (L).

Source of variation	d.f.		NaCl - stress levels g/l														
			Plant regeneration (%)					Green plant regeneration (%)					Albino plant regeneration (%)				
	S	Comb.	Control	4	8	12	Comb.	Control	4	8	12	Comb.	Control	4	8	12	Comb.
Salinity levels(L)	-	3	-	-	-	-	327.43**	-	-	-	-	135.12**	-	-	-	-	41.99**
Genotypes (G)	44	44	4.29**	2.95**	1.86**	1.03**	8.93**	2.88**	2.21**	1.12**	0.57**	5.96**	0.69**	0.41**	0.26	0.13	0.93**
Parents (P)	8	8	5.58**	3.44**	1.71**	0.90**	27.13**	3.51**	2.02**	0.96**	0.55*	14.52**	0.53**	0.51*	0.17	0.08	2.71**
Crosses (C)	35	35	3.23**	2.57**	1.74**	1.06**	33.42**	2.66**	2.22**	1.14**	0.58**	16.61**	0.45**	0.34*	0.24	0.14	4.52**
P *vs*. C	1	1	31.21**	12.17**	7.48**	1.08*	41.25**	5.56**	3.56**	1.87**	0.14	9.32**	10.43**	2.28**	1.87**	0.40	11.36**
G × L	-	132					0.40**					0.30**					0.18
P × L	-	24					0.49**					0.34*					0.09
C × L	-	105					0.29**					0.28**					0.18
P *vs*. C × L	-	3	-	-	-	-	3.56**	-	-	-	-	0.72*	-	-	-	-	1.17**
Error	405	1620	0.18	0.22	0.19	0.21	0.20	0.20	0.18	0.20	0.22	0.20	0.15	0.22	0.25	0.18	0.19

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

The callus derived from anthers was subcultured on MS medium. When the calli were placed onto MS medium containing on different concentrations of salinity stress (control, 4, 8 and 12 g/L NaCl), some of calli differentiated into embryoids. However, many of calli did not differentiate and some of calli differentiated into shoots. Plant regeneration of the callus was dependent on the genotype and the culture medium employed. Effect of genotypes and NaCl concentrations on the frequency of plant regeneration was presented in Table (3). Results showed that the two crosses (P3 × P9 and P1 × P3) produced the highest mean values of plant regeneration (31.39% and 29.72% respectively), while the variety; Sids-14 (P6), produced the lowest values (7.22%). The control medium (NaCl free medium) gave better response to plant regeneration as compared to the other media containing 12 g/L of NaCl. The percentage of plant regeneration from the

control medium was 29.80%, while the medium containing 12 g/l of NaCl gave the lowest one across all genotypes (7.75%). (Table 3 and Figure 2).

Table (3): Effect of salinity levels on plant regeneration and regenerated green plant response of 36 F1 wheat crosses and their respective parents.

Genotypes	NaCl -stress levels (g/l)									
	Plant regeneration (%)					Green plant regeneration (%)				
	Control	4	8	12	Comb.	Control	4	8	12	Comb.
Gemmeiza -7 (P_1)	27.78	18.89	12.22	7.78	16.67	22.22	14.44	8.89	6.67	13.06
Gemmeiza - 9 (P_2)	26.67	17.78	10.00	5.56	15.00	15.56	11.11	5.56	3.33	8.89
Gemmeiza -11 (P3)	35.56	26.67	16.67	11.11	22.50	25.56	15.56	11.11	7.78	15.00
Giza -171 (P_4)	13.33	10.00	6.67	3.33	8.33	8.89	6.67	4.44	2.22	5.56
Sakha - 95 (P_5)	16.67	13.33	8.89	4.44	10.83	11.11	8.89	6.67	3.33	7.50
Sids -14 (P_6)	12.22	8.89	5.56	2.22	7.22	7.78	4.44	3.33	1.11	4.17
Misr-3 (P_7)	24.44	16.67	10.00	6.67	14.44	18.89	13.33	7.78	5.56	11.39
Shandaweel-1 (P_8)	25.56	17.78	11.11	6.67	15.28	16.67	12.22	6.67	4.44	10.00
Line-Azhar-1 (P_9)	33.33	27.78	20.00	12.22	23.33	24.44	21.11	14.44	8.89	17.22
$P_1 \times P_2$	37.78	27.78	18.89	10.00	23.61	26.67	20.00	11.11	6.67	16.11
$P_1 \times P_3$	44.44	33.33	24.44	16.67	29.72	33.33	27.78	18.89	12.22	23.06
$P_1 \times P_4$	31.11	23.33	11.11	3.33	17.22	16.67	12.22	6.67	2.22	9.44
$P_1 \times P_5$	35.56	25.56	16.67	8.89	21.67	25.56	18.89	10.00	5.56	15.00
$P_1 \times P_6$	25.56	17.78	10.00	4.44	14.44	13.33	7.78	5.56	3.33	7.50
$P_1 \times P_7$	40.00	31.11	21.11	14.44	26.67	30.00	24.44	15.56	10.00	20.00
$P_1 \times P_8$	37.78	25.56	18.89	10.00	23.06	27.78	20.00	13.33	7.78	17.22
$P_1 \times P_9$	38.89	27.78	20.00	12.22	24.72	28.89	21.11	14.44	8.89	18.33
$P_2 \times P_3$	34.44	17.78	13.33	5.56	17.78	18.89	10.00	6.67	4.44	10.00
$P_2 \times P_4$	32.22	20.00	11.11	4.44	16.94	17.78	14.44	7.78	2.22	10.56
$P_2 \times P_5$	27.78	17.78	13.33	6.67	16.39	16.67	12.22	8.89	5.56	10.83
$P_2 \times P_6$	26.67	16.67	10.00	4.44	14.44	14.44	7.78	4.44	2.22	7.22
$P_2 \times P_7$	30.00	17.78	13.33	5.56	16.67	16.67	11.11	7.78	3.33	9.72
$P_2 \times P_8$	26.67	15.56	12.22	6.67	15.28	15.56	8.89	5.56	3.33	8.33
$P_2 \times P_9$	33.33	22.22	17.78	7.78	20.28	21.11	14.44	10.00	5.56	12.78
$P_3 \times P_4$	35.56	25.56	18.89	12.22	23.06	25.56	17.78	12.22	8.89	16.11
$P_3 \times P_5$	28.89	21.11	15.56	6.67	18.06	16.67	12.22	8.89	4.44	10.56
$P_3 \times P_6$	36.67	27.78	20.00	10.00	23.61	20.00	15.56	13.33	6.67	13.89
$P_3 \times P_7$	33.33	24.44	16.67	8.89	20.83	18.89	14.44	8.89	5.56	11.94
$P_3 \times P_8$	32.22	25.56	17.78	11.11	21.67	22.22	16.67	11.11	6.67	14.17
$P_3 \times P_9$	46.67	35.56	25.56	17.78	31.39	31.11	25.56	16.67	11.11	21.11
$P_4 \times P_5$	33.33	24.44	14.44	6.67	19.72	21.11	13.33	8.89	3.33	11.67
$P_4 \times P_6$	28.89	21.11	13.33	5.56	17.22	15.56	12.22	6.67	4.44	9.72
$P_4 \times P_7$	32.22	25.56	16.67	6.67	20.28	18.89	15.56	10.00	5.56	12.50
$P_4 \times P_8$	31.11	24.44	17.78	11.11	21.11	23.33	16.67	13.33	7.78	15.28
$P_4 \times P_9$	21.11	15.56	7.78	4.44	12.22	12.22	8.89	4.44	2.22	6.94
$P_5 \times P_6$	30.00	20.00	14.44	8.89	18.33	20.00	14.44	13.33	7.78	13.89
$P_5 \times P_7$	32.22	26.67	16.67	10.00	21.39	21.11	16.67	12.22	5.56	13.89
$P_5 \times P_8$	20.00	13.33	6.67	3.33	10.83	11.11	8.89	4.44	2.22	6.67
$P_5 \times P_9$	26.67	18.89	12.22	6.67	16.11	15.56	11.11	7.78	3.33	9.44
$P_6 \times P_7$	23.33	16.67	10.00	4.44	13.61	14.44	10.00	6.67	2.22	8.33
$P_6 \times P_8$	25.56	15.56	8.89	5.56	13.89	15.56	8.89	5.56	4.44	8.61
$P_6 \times P_9$	20.00	12.22	7.78	4.44	11.11	13.33	7.78	4.44	3.33	7.22
$P_7 \times P_8$	28.89	17.78	12.22	6.67	16.39	17.78	12.22	7.78	4.44	10.56
$P_7 \times P_9$	34.44	26.67	17.78	11.11	22.50	26.67	21.11	11.11	6.67	16.39
$P_8 \times P_9$	22.22	16.67	10.00	5.56	13.61	13.33	10.00	5.56	3.33	8.06
Mean	29.80	21.19	14.10	7.75		19.31	13.98	9.09	5.26	
L.S.D. 0.05 L.S.D. 0.01	Plant regeneration					Green plant regeneration				
NaCl levels 0.05 (L) 0.01	-	-	-	-	0.059 0.077	-	-	-	-	0.058 0.077
Genotype 0.05 (G) 0.01	0.371 0.489	0.410 0.539	0.386 0.508	0.403 0.530	0.197 0.258	0.388 0.511	0.370 0.487	0.393 0.518	0.411 0.541	0.196 0.258
Interaction 0.05 (G×L) 0.01	-	-	-	-	0.393 0.517	-	-	-	-	0.392 0.515

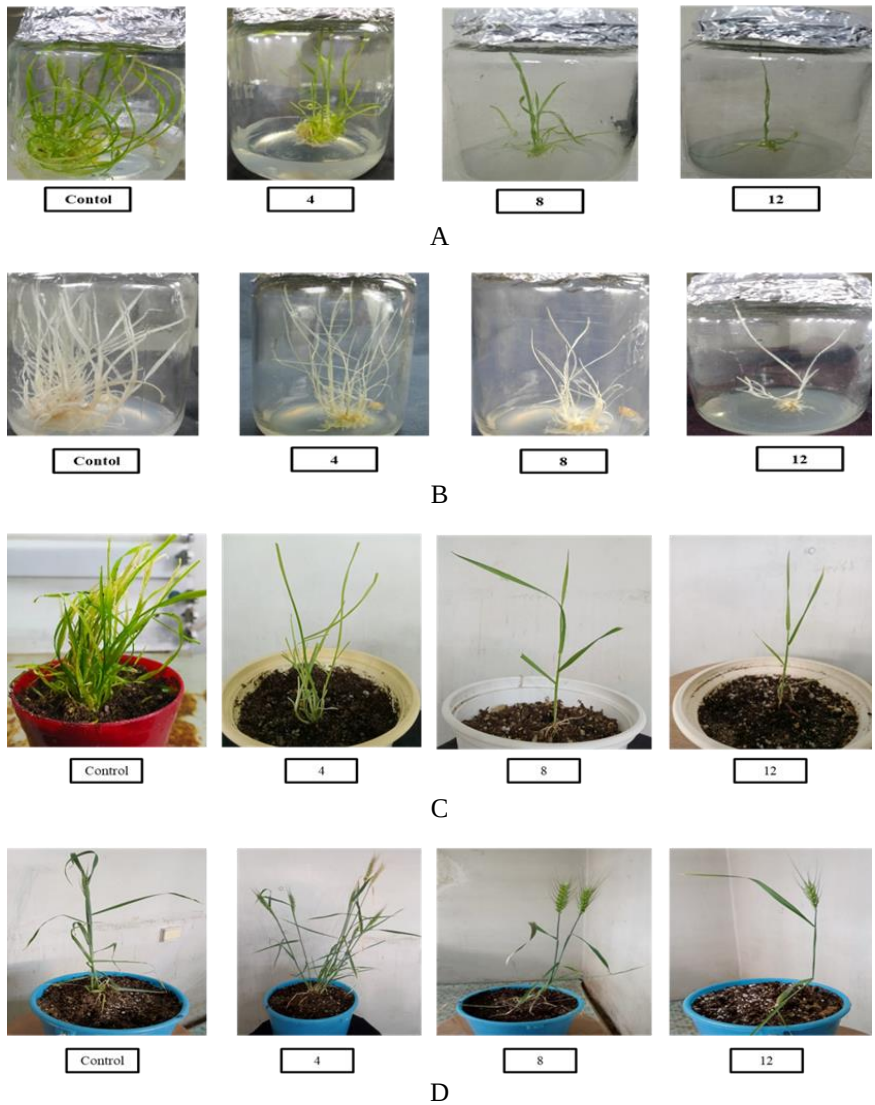

Figure (2): *In vitro* induction of haploid wheat plants through callus culture and their subsequent transfer to pots under NaCl - stress treatments (0, 4, 8 and 12 g/l). A -B) Green and albino plantlets emerging from cultured callus. C) Plant transplanted from the jar to small pot with mixture of soil, sand and compost in greenhouse. D) Regenerated plants under greenhouse conditions.

Results presented in Table (3) indicated strong interaction between genotypes and concentrations of NaCl. The highest response to regenerated plants frequency was recorded for the cross (P3 × P9), when their cultures were grown on the control (46.67%), while the lowest response was recorded for the variety;

Sids-14 (P6) on the medium containing 12 g/L of NaCl (2.22%). These results demonstrated the efficiency of the regeneration medium used. Effect of genotype on plant regeneration from callus cultures have been reported previously (Binte Mostafiz and Wagiran, 2018; Dornelles *et al*., 1997; Khatun *et al*., 2010; Kowalska and Arseniuk, 2016). Dornelles *et al*. (1997) and El-Hennawy *et al*. (2011) reported that plant regeneration from callus was found to be highly heritable. Genetic control of plant regeneration has been observed in several plant species and exploited in some cases to improve plant materials for use in tissue culture research including anther culture (Khatun *et al*., 2010; Morshed *et al*., 2014). Results in Table (3) showed that plant regeneration ability of all the tested genotypes was decreased significantly with increasing concentrations of NaCl in selective media. The tested genotypes differed in their ability to regenerate plants after exposure to NaCl concentrations (4, 8 and 12 g/L of NaCl). The two crosses (P1 × P3 and P3 × P9) were most able to do this and were therefore the most tolerant genotypes with parent that exhibited very good tolerance, whereas the two crosses P1 × P4 and P5 × P8 had a high sensitivity response, especially at the highest stress level of 12 g/L NaCl (Table 3). The typical decrease in plant regeneration of crop plants in response to salt stress is due to salt shortage in the cells, which leads to a decrease in cell turgor and eventually cell growth. Addition of NaCl in culture media lowers water potential of the medium that affect cell division leading to reduced callus growth and consequently influences regeneration (Abdel-Hady, 2006; Munir and Aftab, 2009). Germana (2011) reported that the exploitation of haploid and DHs as a powerful breeding tool requires the availability of reliable tissue culture protocols that can overcome several methodology problems, such as low frequencies of embryo induction, albinism, plant regeneration, plant survival and the genotype-dependent response, in order to improve the regeneration efficiency in a wider range of genotypes. He also reported that there is no single standard condition or protocol for inducing pollen-derived plant formation. In the present investigation, the presence of significant variation in plant regeneration due to genotypes, concentrations of NaCl and their interactions were observed. The frequencies of green plantlets achieved of nine parents and their crosses studied under the four NaCl concentrations are presented in Table (3). Results showed that the three crosses (P1 × P3, P3 × P9 and P1 × P7) produced the highest mean values of green plantlets (23.06, 21.11 and 20.00% respectively) compared to the parental Line-Azhar-1 (P9) and Gemmeiza-11 (P3), which gave (17.22% and 15.00% respectively) while the two varieties Giza-171 (P4), Sids-14 (P6) produced the lowest values (4.17% and 5.56%, respectively). The control (NaCl - free medium) gave better response to green plantlets as compared to the other media containing 4, 8 and 12 g/l of NaCl. The

percentage of green plantlets from the control was 19.31%. In contrast, the medium containing 12 g/l of NaCl gave the lowest one (5.26%) across all genotypes (Table 3). The interaction between concentrations of NaCl and genotypes was highly significant (Table 3). The highest frequency of green plantlets in the control was recorded for the cross (P1 × P3). On the contrary, the lowest number of green plantlets was observed for the variety Sids-14 (P6) under the highest stress level 12 g/l of NaCl. These results indicated that frequency of calli capable of producing green plantlets was affected by genotypes. Similar results were obtained by Ascough *et al*., (2006) and El-Hennawy *et al*., (2011), who found genotypic effect on green plant regeneration in wheat. As pointed out in different investigations, androgenic response in wheat was a heritable trait and can be transferred into agriculturally desirable material by crossing (Al-Ashkar, 2013; Foroughi-wehr *et al*., 1982; Tuvesson *et al*., 2000). The occurrence of albino plantlets, which is usually uncounted in most similar studies, composes a major problem for the application of anther culture technique in wheat breeding programs. Results in Table (4) showed that the percentage of albino plantlets ranged from 2.78% for the variety; Giza-171 (P4) to 10. 28% for the cross (P3 × P9) among the genotypes across the four different concentrations of NaCl (Table 4). The response of albino plantlets varied according to different concentrations used, indicating that the control (NaCl - free medium) gave the highest mean value of albino plantlets (10.49%). On the other hand, the medium containing 12 g/L of NaCl gave the lowest one (2.49%) across all genotypes. Furthermore, three genotypes (P3 × P6, P2 × P3 and P3 × P9) gave the highest percentage of albino plantlets (16.67% and 15.56 respectively) in control, while the fifteen genotypes gave the lowest one (1.11%) under the stress level of 12 g/L NaCl (Table 4). Similar results were found by Hassawi *et al*., (2005) who observed genotypic effect on albino plant regeneration. Andersen *et al*. (1988), Kumari *et al*. (2009), Nielsen *et al*. (2015), and Zhao *et al*. (2017) stated that the formation of albino plantlets was genetically and environmentally controlled. In addition to the effect of genotype and the duration of maintaining calli in culture, the development stage of microspore at inoculation time as well as the chloroplast DNA deletions may affect occurrence of albino plantlets (Liang *et al*., 1990). Liu *et al*. (2002), Caredda *et al*. (2004), Esteves and Belzile (2014), El-Goumi *et al*. (2017), and Gajecka *et al*. (2020) reported that albino plantlets in wheat and barley have altered plastids in which the DNA has been changed or partially deleted under stress treatment *in vitro*. Hentour *et al*. (2020) stated that it was probably due to the interference of NaCl in proplastid biosynthesis during morphogenesis. The results of the present study are in accordance with previous reports of Zamani *et al*. (2003), and El-Hennawy *et al*. (2011), who indicated that the

genotype played an important role in anther culture. Furthermore, the two crosses (P1 × P3 and P3 × P9) with the highest response in anther culture had parent that exhibited very good response under the four stress levels.

Table (4): Effect of salinity levels on albino plant regeneration response of 36 F_1 wheat crosses and their respective parents.

Genotypes	NaCl -stress levels (g/l)				
	Albino plant regeneration (%)				
	Control	4	8	12	Comb.
Gemmeiza -7 (P_1)	5.56	4.44	3.33	1.11	3.61
Gemmeiza - 9 (P_2)	11.11	6.67	4.44	2.22	6.11
Gemmeiza -11 (P3)	10.00	11.11	5.56	3.33	7.50
Giza -171 (P_4)	4.44	3.33	2.22	1.11	2.78
Sakha - 95 (P_5)	5.56	4.44	2.22	1.11	3.33
Sids -14 (P_6)	4.44	4.44	2.22	1.11	3.06
Misr-3 (P_7)	5.56	3.33	2.22	1.11	3.06
Shandaweel-1 (P_8)	8.89	5.56	4.44	2.22	5.28
Line-Azhar-1 (P_9)	8.89	6.67	5.56	3.33	6.11
$P_1 \times P_2$	11.11	7.78	7.78	3.33	7.50
$P_1 \times P_3$	11.11	5.56	5.56	4.44	6.67
$P_1 \times P_4$	14.44	11.11	4.44	1.11	7.78
$P_1 \times P_5$	10.00	6.67	6.67	3.33	6.67
$P_1 \times P_6$	12.22	10.00	4.44	1.11	6.94
$P_1 \times P_7$	10.00	6.67	5.56	4.44	6.67
$P_1 \times P_8$	10.00	5.56	5.56	2.22	5.83
$P_1 \times P_9$	10.00	6.67	5.56	3.33	6.39
$P_2 \times P_3$	15.56	7.78	6.67	1.11	7.78
$P_2 \times P_4$	14.44	5.56	3.33	2.22	6.39
$P_2 \times P_5$	11.11	5.56	4.44	1.11	5.56
$P_2 \times P_6$	12.22	8.89	5.56	2.22	7.22
$P_2 \times P_7$	13.33	6.67	5.56	2.22	6.94
$P_2 \times P_8$	11.11	6.67	6.67	3.33	6.94
$P_2 \times P_9$	12.22	7.78	7.78	2.22	7.50
$P_3 \times P_4$	10.00	7.78	6.67	3.33	6.94
$P_3 \times P_5$	12.22	8.89	6.67	2.22	7.50
$P_3 \times P_6$	16.67	12.22	6.67	3.33	9.72
$P_3 \times P_7$	14.44	10.00	7.78	3.33	8.89
$P_3 \times P_8$	10.00	8.89	6.67	4.44	7.50
$P_3 \times P_9$	15.56	10.00	8.89	6.67	10.28
$P_4 \times P_5$	12.22	11.11	5.56	3.33	8.06
$P_4 \times P_6$	13.33	8.89	6.67	1.11	7.50
$P_4 \times P_7$	13.33	10.00	6.67	1.11	7.78
$P_4 \times P_8$	7.78	7.78	4.44	3.33	5.83
$P_4 \times P_9$	8.89	6.67	3.33	2.22	5.28
$P_5 \times P_6$	10.00	5.56	1.11	1.11	4.44
$P_5 \times P_7$	11.11	10.00	4.44	4.44	7.50
$P_5 \times P_8$	8.89	4.44	2.22	1.11	4.17
$P_5 \times P_9$	11.11	7.78	4.44	3.33	6.67
$P_6 \times P_7$	8.89	6.67	3.33	2.22	5.28
$P_6 \times P_8$	10.00	6.67	3.33	1.11	5.28
$P_6 \times P_9$	6.67	4.44	3.33	1.11	3.89
$P_7 \times P_8$	11.11	5.56	4.44	2.22	5.83
$P_7 \times P_9$	7.78	5.56	6.67	4.44	6.11
$P_8 \times P_9$	8.89	6.67	4.44	2.22	5.56
Mean	10.49	7.21	5.01	2.49	
L.S.D. 0.05 L.S.D. 0.01	Albino plant regeneration				
NaCl levels 0.05 (L) 0.01	-	-	-	-	0.058 0.076
Genotype 0.05 (G) 0.01	0.336 0.442	0.408 0.537	0.435 0.573	0.372 0.490	0.195 0.257
Interaction 0.05 (G × L) 0.01	-	-	-	-	0.390 0.513

These results indicate that one high responding parent could be used to generate responding F1 hybrids, although there is no guarantee of a high response in the hybrids because the inheritance of an anther culture response may be more complicated (Masojc *et al*., 1993). Zamani *et al*. (2003) also reported that a well-responding parent could lead to the production of sufficient green plants for breeding purposes. In addition, the data obtained from this study indicate that hybrids originating from one parent with very good or intermediate performance in anther culture would be of value for developing an *in-vitro* system with a high production of green plants under the salinity stress conditions.

3.3 General and specific combining abilities for all in vitro studied traits under salt stress treatments in wheat

Information on the inheritance or combining ability of plant regeneration in anther culture is of great importance in an attempt to increase the efficiency of anther culture (Can and Yoshida, 1999). Hou *et a1*. (1994), and Zamani *et al*. (2003) reported that by understanding the inheritance patterns of anther culture response, breeders could improve the procedures by crossing highly responsive with non-responsive genotypes and could predict the level of response of the hybrids or optimize the allocation of resources for doubled haploid production. The analysis of variance for combining ability of different concentrations of sodium chloride (NaCl) for all *in vitro* studied traits is presented in Table (5). Results revealed that variances due to general (GCA) combining abilities were significant or highly significant for all in vitro traits tested under the four stress levels as well as the combined analysis except albino plant regeneration at the stress level of 12 g/L NaCl. Moreover, variances due to specific (SCA) combining abilities were significant or highly significant for all *in vitro* traits tested under the four stress levels and the combined analysis except albino plant regeneration at the two stress levels of 8 and 12 g/L NaCl.

Table (5): Mean squares of single (S) and combined (Comb) analysis for general and specific combining abilities of F1 wheat crosses and their parents for the in vitro studied traits under four salinity levels (L).

Source of variation	d.f.		NaCl - stress levels g/l														
	S	Comb.	Plant regeneration (%)					Green plant regeneration (%)					Albino plant regeneration (%)				
			Control	4	8	12	Comb.	Control	4	8	12	Comb.	Control	4	8	12	Comb.
GCA	8	8	1.118**	0.750**	0.456**	0.279**	0.591**	0.832**	0.595**	0.242**	0.147**	0.399**	0.090**	0.052*	0.056*	0.026	0.040**
SCA	36	36	0.276**	0.193**	0.126**	0.064**	0.141**	0.167**	0.139**	0.084**	0.037*	0.092**	0.064**	0.039**	0.019	0.011	0.020**
GCA × L	-	24	-	-	-	-	0.298**	-	-	-	-	0.195**	-	-	-	-	0.031**
SCA × L	-	108	-	-	-	-	0.081**	-	-	-	-	0.055**	-	-	-	-	0.016**
Error	405	1620	0.018	0.022	0.019	0.021	0.005	0.020	0.018	0.020	0.022	0.004	0.015	0.022	0.025	0.018	0.005
GCA/SCA	-	-	4.050	3.886	3.619	4.395	4.191	4.982	4.280	2.880	3.972	4.336	1.406	1.333	2.947	2.363	2.000
(GCA × L) / GCA	-	-	-	-	-	-	0.504	-	-	-	-	0.488	-	-	-	-	0.775
(SCA × L) / SCA	-	-	-	-	-	-	0.574	-	-	-	-	0.597	-	-	-	-	0.800

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

These results indicate the importance of both additive and non-additive gene action in the expression of these traits. However, the ratio of GCA/SCA was found more than unity for all in vitro studied traits under the four stress levels revealing the predominant role of additive gene action in the inheritance of these traits. Yildirim *et al*. (2008), and Kalhoro *et al*. (2015) have also reported high GCA/SCA ratios for all *in vitro* traits in wheat. On the other hand, He *et al*. (2006), Al-Ashkar (2014), and Kalhoro *et al*. (2015), found that general combining ability variances were less than specific combining ability ones for green plant regeneration in wheat. The mean squares of interaction between NaCl stress levels and both general and specific combining abilities were also highly significant for all in vitro studied traits, indicating that magnitudes of different types of gene action were fluctuated from one stress level to another. It is fairly evident that the ratio of SCA × NaCl stress levels / SCA was higher than ratio of GCA × NaCl stress levels / GCA for all traits studied. These results indicated that non-additive genetic effects were much more influenced by the salinity stress levels than additive genetic effects in these traits. Such results indicate that NaCl stress treatments are considered as an effective factor for declaring GCA and SCA. Thus, the breeder should utilize the appropriate breeding method under each salt stress for developing desired wheat genotypes.

Table (6): Estimates of general combining ability effects of nine wheat parents tested for the *in vitro* studied traits under four salinity levels.

Source of variation	d.f.		NaCl - stress levels g/l														
	S	Comb.	Plant regeneration (%)					Green plant regeneration (%)					Albino plant regeneration (%)				
			Control	4	8	12	Comb.	Control	4	8	12	Comb.	Control	4	8	12	Comb.
Gemmeiza -7 (P1)	0.398**	0.312**	0.201**	0.147**	0.424**	0.340**	0.184**	0.137**	-0.026	-0.030	0.017	0.012	0.398**	0.312**	0.201**	0.147**	0.424**
Gemmeiza - 9 (P2)	0.034	-0.170**	-0.090*	-0.125**	-0.112**	-0.151**	-0.143**	-0.099*	0.146**	-0.021	0.054	-0.024	0.034	-0.170**	-0.090*	-0.125**	-0.112**
Gemmeiza -11 (P3)	0.534**	0.430**	0.365**	0.275**	0.370**	0.259**	0.229**	0.192**	0.165**	0.170**	0.135**	0.085*	0.534**	0.430**	0.365**	0.275**	0.370**
Giza -171 (P4)	-0.211**	-0.097*	-0.135**	-0.134**	-0.194**	-0.123**	-0.098*	-0.090*	-0.017	0.024	-0.037	-0.042	-0.211**	-0.097*	-0.135**	-0.134**	-0.194**
Sakha - 95 (P5)	-0.247**	-0.142**	-0.108**	-0.089*	-0.185**	-0.123**	-0.025	-0.063	-0.063	-0.021	-0.083	-0.024	-0.247**	-0.142**	-0.108**	-0.089*	-0.185**
Sids -14 (P6)	-0.466**	-0.379**	-0.290**	-0.207**	-0.412**	-0.378**	-0.198**	-0.144**	-0.054	-0.003	-0.092*	-0.070	-0.466**	-0.379**	-0.290**	-0.207**	-0.412**
Misr-3 (P7)	0.043	0.067	0.028	0.029	0.079*	0.095*	0.038	0.019	-0.035	-0.030	-0.010	0.012	0.043	0.067	0.028	0.029	0.079*
Shandaweel-1 (P8)	-0.184**	-0.179**	-0.117**	-0.034	-0.103**	-0.105**	-0.089*	-0.035	-0.081*	-0.076	-0.028	-0.006	-0.184**	-0.179**	-0.117**	-0.034	-0.103**
Line-Azhar-1 (P9)	0.098*	0.158**	0.146**	0.138**	0.133**	0.186**	0.102*	0.083*	-0.035	-0.012	0.044	0.058	0.098*	0.158**	0.146**	0.138**	0.133**
SE (gi)	0.038	0.042	0.040	0.041	0.040	0.038	0.040	0.042	0.034	0.042	0.045	0.038	0.038	0.042	0.040	0.041	0.040
SE (gi- gj)	0.057	0.063	0.059	0.062	0.060	0.057	0.061	0.063	0.052	0.063	0.067	0.057	0.057	0.063	0.059	0.062	0.060

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

Similar results were obtained by Gholizadeh *et al*. (2018). Estimates of general combining ability effects for each parent under NaCl stress treatments are presented in Table (6). Results showed that the Gemmeiza-7 (P1) and Gemmeiza-11 (P3) followed by Line-Azhar-1 (P9) were the best combiners for plant regeneration and regenerated green plantlets under varying concentrations of NaCl due to their significant or highly significant positive GCA values, revealing the great value of such parents as promising progenitors for high plant regeneration and green plant regeneration. On the contrary, the other four varieties; Giza-171 (P4), Sakha-95 (P5), Sids-14 (P6) and Shandaweel-1 (P8) showed negative general combining ability effects for plant regeneration and regenerated green plantlets under salinity stress conditions. For albino plant regeneration, highly significant positive general combining ability effects were detected for

Gemmeiza-11 (P3) under the four stress levels. On the other hand, some varieties Sakha-95 (P5), Sids-14 (P6) and Shandaweel-1 (P8) had poor general combiners as they showed negative GCA effects in the four stress treatments. Specific combining ability effects (SCA) for each cross are shown in Table (7).

Table (7): Estimates of specific combining ability effects of 36 F_1 wheat crosses tested for the *in vitro* studied traits under four salinity levels.

Crosses	NaCl -stress levels (g/l)											
	Plant regeneration (%)				Green plant regeneration (%)				Albino plant regeneration (%)			
	Control	4	8	12	Control	4	8	12	Control	4	8	12
$P_1 \times P_2$	0.285*	0.451**	0.320*	0.180	0.355**	0.354**	0.142	0.093	-0.069	0.098	0.178	0.085
$P_1 \times P_3$	0.385**	0.351**	0.365**	0.380**	0.473**	0.645**	0.469**	0.302*	-0.087	-0.293*	-0.104	0.076
$P_1 \times P_4$	-0.069	-0.022	-0.335**	-0.411**	-0.464**	-0.373**	-0.304*	-0.316*	0.395**	0.353**	-0.031	-0.096
$P_1 \times P_5$	0.367**	0.224	0.138	0.044	0.327*	0.227	-0.076	-0.044	0.040	-0.002	0.215	0.085
$P_1 \times P_6$	-0.315*	-0.240	-0.280*	-0.238	-0.545**	-0.518**	-0.304*	-0.262	0.231*	0.280*	0.024	0.031
$P_1 \times P_7$	0.476**	0.515**	0.402**	0.425**	0.464**	0.509**	0.360**	0.275*	0.013	0.007	0.042	0.149
$P_1 \times P_8$	0.504**	0.260	0.347**	0.089	0.445**	0.309*	0.287*	0.129	0.058	-0.047	0.060	-0.033
$P_1 \times P_9$	0.322**	0.124	0.184	0.116	0.109	0.118	0.196	0.111	0.213	-0.011	-0.013	0.004
$P_2 \times P_3$	-0.151	-0.567**	-0.344**	-0.347**	-0.291*	-0.464**	-0.304*	-0.162	0.140	-0.102	-0.040	-0.187
$P_2 \times P_4$	0.395**	0.160	-0.044	-0.038	0.173	0.318**	0.124	-0.080	0.222*	-0.156	-0.167	0.040
$P_2 \times P_5$	0.031	0.005	0.129	0.116	0.064	0.118	0.151	0.193	-0.033	-0.111	-0.022	-0.078
$P_2 \times P_6$	0.149	0.142	0.011	0.035	0.091	-0.027	-0.076	-0.025	0.058	0.171	0.087	0.067
$P_2 \times P_7$	-0.060	-0.204	-0.007	-0.102	-0.200	-0.200	-0.013	-0.089	0.140	-0.002	0.005	-0.015
$P_2 \times P_8$	-0.133	-0.158	0.038	0.062	-0.118	-0.200	-0.085	-0.035	-0.015	0.044	0.124	0.104
$P_2 \times P_9$	0.185	0.105	0.275*	-0.011	0.145	0.009	0.124	0.047	0.040	0.080	0.151	-0.060
$P_3 \times P_4$	0.195	0.060	0.202	0.262*	0.391**	0.209	0.151	0.229	-0.196	-0.147	0.051	0.031
$P_3 \times P_5$	-0.369**	-0.295*	-0.125	-0.284*	-0.418**	-0.291*	-0.222	-0.198	0.049	-0.002	0.096	-0.087
$P_3 \times P_6$	0.549**	0.542**	0.456**	0.135	0.109	0.264*	0.351**	0.084	0.440**	0.280*	0.105	0.058
$P_3 \times P_7$	-0.260*	-0.204	-0.162	-0.202	-0.482**	-0.309*	-0.285*	-0.180	0.222*	0.107	0.124	-0.024
$P_3 \times P_8$	-0.133	0.142	0.084	0.062	0.000	0.091	0.042	-0.025	-0.133	0.053	0.042	0.095
$P_3 \times P_9$	0.885**	0.705**	0.520**	0.489**	0.564**	0.600**	0.351**	0.256	0.322**	0.089	0.169	0.231
$P_4 \times P_5$	0.776**	0.533**	0.275*	0.125	0.545**	0.191	0.105	-0.016	0.231*	0.344*	0.169	0.140
$P_4 \times P_6$	0.595**	0.469**	0.356**	0.144	0.273*	0.345**	0.078	0.165	0.322**	0.125	0.278	-0.015
$P_4 \times P_7$	-2.515**	-1.876**	-1.162**	-0.593**	-1.618**	-1.227**	-0.758**	-0.398**	-0.896**	-0.647**	-0.404**	-0.196
$P_4 \times P_8$	0.513**	0.569**	0.584**	0.471**	0.664**	0.473**	0.569**	0.356**	-0.151	0.098	0.015	0.122
$P_4 \times P_9$	-2.569**	-1.967**	-1.280**	-0.702**	-1.673**	-1.318**	-0.822**	-0.462**	-0.896**	-0.665**	-0.458**	-0.242*
$P_5 \times P_6$	0.731**	0.415**	0.429**	0.398**	0.664**	0.545**	0.605**	0.438**	0.067	-0.129	-0.176	-0.033
$P_5 \times P_7$	0.422**	0.569**	0.311*	0.262*	0.273*	0.173	0.269*	0.075	0.149	0.398**	0.042	0.185
$P_5 \times P_8$	-0.451**	-0.385**	-0.444**	-0.275*	-0.445**	-0.227	-0.304*	-0.171	-0.005	-0.156	-0.140	-0.096
$P_5 \times P_9$	-0.133	-0.222	-0.207	-0.147	-0.282*	-0.318**	-0.195	-0.189	0.149	0.080	-0.013	0.040
$P_6 \times P_7$	-0.160	-0.095	-0.107	-0.120	-0.100	-0.073	-0.058	-0.144	-0.060	-0.020	-0.049	0.031
$P_6 \times P_8$	0.267*	0.051	-0.062	0.044	0.182	0.027	-0.031	0.011	0.085	0.025	-0.031	-0.051
$P_6 \times P_9$	-0.515**	-0.585**	-0.425**	-0.229	-0.255*	-0.364**	-0.322*	-0.107	-0.260*	-0.238	-0.104	-0.115
$P_7 \times P_8$	0.058	-0.195	-0.080	-0.093	-0.109	-0.145	-0.067	-0.053	0.167	-0.047	-0.013	-0.033
$P_7 \times P_9$	0.276*	0.269*	0.156	0.135	0.455**	0.364**	0.042	0.029	-0.178	-0.111	0.115	0.104
$P_8 \times P_9$	-0.596**	-0.385**	-0.398**	-0.302*	-0.564**	-0.436**	-0.331*	-0.216	-0.033	0.035	-0.067	-0.078
SE (sij)	0.123	0.135	0.127	0.133	0.128	0.122	0.130	0.136	0.111	0.135	0.144	0.123
SE (sij- sik)	0.181	0.199	0.188	0.196	0.189	0.180	0.191	0.200	0.163	0.199	0.212	0.181

Results indicated that 10 out of 36 crosses showed significant or highly significant desirable specific combining ability effects for plant regeneration under the four stress levels (control, 4, 8 and 12 g/l NaCl) except the four crosses P1 × P2, P3 × P6, P4 × P5 and P4 × P6 in the stress level of 12 g/l. They included three types of combinations; high × high, high × low and low × low general combining ability effects. The four crosses; P1 × P7, P3 × P9, P4 × P8 and P5 × P6 recorded the highest SCA effects for plant regeneration under the four stress levels. The two crosses; P1 × P7 and P3 × P9 involved at least one parent as good general combiner. Therefore, these crosses could be of great value for a wheat plant regeneration

selection system in breeding programs. For green plant regeneration, significant or highly significant and positive SCA effects were detected in 6 out of 36 F1 crosses under the four salinity stress levels except the two crosses P1 × P8 and P3 × P9 at the stress level of 12 g/l. In contrast, negative specific combining ability effects were observed in fifteen crosses for this trait under the four stress levels. It is worthy to note that it is not necessary that parents having high estimates of GCA effects would also give high estimates of SCA effects. In the cross (P3 × P7) for regenerated green plant under the control and the stress level of 4 g/l NaCl, both parents involved had high GCA effects, but gave comparatively very low SCA effects. El-Hennawy *et al*. (2018) found that low SCA effects in such cases might be attributed to some internal cancellation of favourable factors or to genetic similarity of the involved parents. On the contrary, the two crosses P4 × P8 and P5 × P6 involving parents with very low GCA effects for plant regeneration and regenerated green plant, recorded high SCA effects for these traits, which might be due to high genetic diversity among the parents. Moreover, the parents having low GCA effects had a relatively high magnitude of non-additive gene effects and thus resulted in high SCA effects when crossed. Regarding albino plant regeneration, negative SCA effects were obtained in five crosses; P2 × P5, P4 × P7, P4 × P9, P5 × P8, and P6 × P9 under the four stress levels. On the contrary, seven crosses; P1 × P6, P1 × P7, P2 × P6, P3 × P6, P3 × P9, P4 × P5, and P5 × P7 gave positive SCA effects for this trait under the salt stress levels. Silva (2010) and Tripathy (2018) reported that quantitative inheritance of anther culture response could complicate the selection process. Combining ability analysis provides a guideline to the breeder in selecting the suitable parents and desirable cross combinations to be used in the formulation of systematic breeding program for the improvement of quantitative characters (Kalhoro *et al*., 2015). Evaluating a good combination depends on both the values of GCA and SCA. A cross combination, which has high SCA value and good parental GCA effects, may be considered as a suitable cross combination. For instance, the two crosses combination P1 × P3 and P3 × P9 are considered to be promosing crosses for varietal improvement purpose, as they showed significant positive SCA effect for plant regeneration under the four stress levels (Table 7) and involved three good general combiner parents (Table 6). In such cases, it would be expected that diverse genes contributing to the better general combining ability effects of the parents are available in the crosses and in the segregating generations, these are likely to give transgressive segregates. Therefore, GCA and SCA effects should be taken into account generally, when developing the strategy of the selection of genotypes for obtaining cross combination with high green plant regeneration ability under salinity stress conditions.

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