

# Genotypic Variation among 117 Doubled Haploids in Agronomic and Yield Attributes under Increased Salinity Levels

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## ABSTRACT

A pot experiment was conducted in 2012/2013 and 2013/2014 seasons to screen 121 wheat (*Triticum aestivum* L.) genotypes (117 doubled haploids derived from a cross between the Egyptian salt tolerant cultivar Sakha-8 and the promising high yielding line L-25, the two parents and two check cultivars) for salt tolerance. A factorial experiment with four salinity levels, i.e. 0, 3000, 6000 and 9000 ppm NaCl was used. The objectives were to find out good donors to improve salt tolerance for Egyptian genotypes and (ii) to determine the superiority of the most tolerant over the most sensitive DH lines or the best check in agronomic and yield attributes. Across seasons, the results indicated that under 6000 and 9000 ppm NaCl salinity conditions, 15 and 61 genotypes, i.e. 12.4 and 50.4 %, respectively could not reach to maturity (not survived). Tillers/ plant trait was the most sensitive, while grain yield was the most tolerant one to both the salinity stresses 9000 and 6000 ppm NaCl. The 10 highest salinity tolerance index (STI) estimates under the three salinity stress treatments 3000, 6000 and 9000 ppm NaCl were shown by L8, L9, L33, L84, L93, L10, L11, L2 and L109, in descending order under all studied salinity stress treatments. The highest yielding and most tolerant DH line (HY-T) under 9000 ppm NaCl was L109 followed by L11, L94, L103, L79 and L81. Under 9000 ppm NaCl, significant higher values were exhibited in HY-T than in low-yielding sensitive (LY-S) by 89.9% for grain yield/plant. The six best DH lines (highest yielding and most tolerant) under 9000 ppm NaCl in this study (L109, L11, L94, L103, L79 and L81) that outyielded the best check cultivar (Sakha-93) by 186.3, 168.1, 160.5, 157.0, 149.8 and 128.5%, respectively, were recommended for field evaluation in Egyptian salt affected areas, hoping that one or more of them could show its superiority in grain yield and salinity tolerance, under the natural salinity conditions, over the best Egyptian cultivars recommended for these areas, such as Sakha-93.

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**Key words:** Bread wheat, Salinity tolerance index, Grain yield, Tillers/plant, Responsive, Efficient, Pot experiment

## Introduction

Although wheat (*Triticum aestivum* L.) productivity in Egypt has increased during the past years, wheat production supplies only 45% of its annual domestic demand. Egypt still is one of the largest countries that import wheat. Wheat imports in 2011 were about 9.8 million tons, with a cost of about 3.2 billion US\$ (FAOSTAT, 2011). Therefore, Egypt needs to make a great effort to increase wheat production. Extending wheat growing outside the Nile Valley is the first effort toward overcoming wheat problems. However, most of the area outside the Nile Valley is affected by salinity; therefore increasing salt tolerance for wheat genotypes is one of the cheap methods to spread growing wheat in these areas.

Wheat is moderately tolerant to salt with threshold without yield loss at 6 dSm<sup>-1</sup> and with yield 50% loss at 13 dSm<sup>-1</sup> (Maas and Hoffmann, 1977). The effect of salinity on tiller and spikelet numbers established during the early vegetative growth stage has a greater influence on final seed yield than the effects exerted on yield components in the latter two phases (Kirby, 1988), indicating the probability of improving salt tolerance of wheat genotypes during early growth stages.

Because breeding for salt tolerance is difficult and of slow progress, many aspects should be considered in a given crop, such as evaluation of a wide range of germplasm to assess the genetic variation. Screening large numbers of genotypes in the field is notoriously difficult because of the variability of salinity within fields (Daniells *et al.*, 2001) and the enormous potential for interaction with other environmental factors, ranging from soil chemical and physical properties to temperature, light flux density and seasonal fluctuation in rainfall. Screening technique has, therefore, often been used

under controlled conditions. Consequently, prediction of field performance is commonly carried out in trial plots method where the salinity of the medium can be readily adjusted to required values (Francios and Mass, 1994). Large numbers of bread wheat genotypes have been screened for salt tolerance in greenhouse; the criteria being biomass production at high salinity (up to 25 mM NaCl) relative to biomass in control conditions (Kingsbury and Epstein, 1984), and a screen by Sayed (1985) of 5000 wheat lines under solution culture, based on survival at high salinity, showed considerable genetic diversity amongst tested genotypes and lines.

Conventional breeding in Egypt was able to develop some bread wheat cultivars, such as Sakha 8 and Sakha 93 of higher salinity tolerance than other commercial cultivars. Genetic diversity in wheat has been reduced mainly due to narrow genetic base of the wheat germplasm (Wei *et al.*, 2002). Therefore wheat breeders are always looking for new methods to enrich breeding material of better tolerance to salinity stress. Using modern biotechnological techniques in plant breeding, could contribute, to a great extent, in the induction of novel genetic variation, which are not existed in the gene pool, such as somaclonal and/or gametoclonal variation (Khan *et al.*, 2001). The anther culture technique helps in developing doubled haploids, in a short time from wheat crosses that show new genetic variation amenable for efficient selection for salinity tolerant genotypes (Mitchell *et al.*, 1992).

One hundred seventeen bread wheat doubled haploid (DH) lines derived from the cross Sakha 8 X Line 25 *via* anther culture technique (Doghma, 2007) were used in the present investigation; the first parent (Sakha 8) of the cross was an Egyptian salinity tolerant cultivar and the second parent (Line 25) was a high yielding promising breeding line. This set of DH lines is expected to include line(s) that accumulated favorable genes for both high-yielding and salinity tolerance. Therefore, the objectives of the present pot experiment were: (i) to screen a large number of wheat doubled haploid lines (117) derived from the cross between the salt tolerant Egyptian cultivar Sakha 8 and the promising high yielding genotype Line-25 *via* anther culture technique in order to find out good donors or traits to improve salt tolerance for Egyptian genotypes through breeding programs and (ii) to determine the superiority of the most tolerant over the most sensitive DH lines or the best check in agronomic and yield attributes.

## Materials and Methods

### Plant materials

Materials used in this study included 117 wheat (*Triticum aestivum* L.) doubled haploid (DH) lines, derived from the cross between Sakha 8 (Egyptian salt tolerant cultivar) and Line 25 (a promising high-yielding breeding line) *via* anther culture technique (Doghma, 2007), the two parents and two check Egyptian cultivars, *i.e.* Sakha 93 and Sids 1. These materials were acquired from Wheat Res. Dept., Field Crop Res. Inst., Agricultural Research Center (ARC), Egypt.

### Sowing method

Five seeds from each of the 121 genotypes were surface sterilized by rinsing in Chlorox solution (35%) for 3 min and washed several times by distilled water and sown in 30 cm pots filled with 5 kg of a mixture of well washed sandy soil for the 1<sup>st</sup> season and well washed mixture of 1 sandy soil and 1 peatmoss (v/v) for the 2<sup>nd</sup> season. A factorial experiment, based on lattice arrangement with two replications was used. Four salinity treatments (0, 3000, 6000, 9000 NaCl ppm) were used. Planting date was on 1<sup>st</sup> and 5<sup>th</sup> of December in 2012/13 and 2013/14 seasons., respectively.

Pots were irrigated with saline solutions of the four NaCl concentrations mixed with full strength Hoagland solution (Hoagland and Arnon, 1950). The irrigation was applied weekly as follows: The 1<sup>st</sup> irrigation (at planting) treatments were as follows: Treatment 1 (0 ppm): Tap water + 0.5g ammonium nitrate + 0.25g monophosphate. Treatment 2 (3000 ppm): Saline solution (3000 ppm NaCl) + 0.5g ammonium nitrate + 0.25g monophosphate. Treatment 3 (6000 ppm): Saline solution (6000 ppm NaCl) + 0.5g ammonium nitrate + 0.25g monophosphate. Treatment 4 (9000 ppm): Saline solution (9000 ppm NaCl) + 0.5g ammonium nitrate + 0.25g monophosphate. The succeeding weekly irrigation treatments were as follows: Treatment 1 (0 ppm): Tap water + Hoagland solution. Treatment 2 (3000 ppm): Saline solution (3000 ppm NaCl + Hoagland solution. Treatment 3 (6000 ppm): Saline solution (6000 ppm NaCl + Hoagland solution. Treatment 4 (9000 ppm): Saline solution (9000 ppm NaCl + Hoagland solution.

Data were recorded on individual plants from each pot as follows: 1. Days to heading (DTH): It is estimated as the number of days from sowing date to the date at which 50% of main spike awns had completely emerged from the flag leaf. 2. Days to maturity (DTM): It is recorded as the number of days from sowing to the date at which 50% of main peduncles have turned to yellow color (physiological maturity). 3. Plant height (PH): It was measured as the height of plant at maturity, measured from the soil surface to level the tip of spike, excluding awns. 4. Number of Tillers/plant (NTPP): It was measured as the total number of tillers per plant as an average of three plants. 5. Number of spikes/plant (NSPP): It was measured as the total number of fertile spikes per plant as an average of three plants. 6. Number of grains/spike (NGPP): It was measured as the total number of grains per main spike as an average of three spikes. 7. Thousand grains weight (TKW): It was measured as the weight of 1000 grains using an electronic balance. 8. Grain yield/plant (GYPP): It was measured as the dry matter (biomass) allocated to the grains per plant as an average of three plants. 9. Straw yield/plant (SYPP): It was measured as the dry matter (biomass) allocated to the straw (the above ground parts of the plant, except grains) as an average of three plants. 10. Biological yield/plant (BYPP): It was measured as the dry matter (biomass) allocated to the grain to the whole plant except root, as an average of the plants. It was estimated as follows: BYPP = GYPP + SYPP. 11. Harvest index (HI): It was estimated as follows: HI = 100 (GYPP / BYPP). The

previously mentioned traits were used to calculate the following parameters: 1. Salinity tolerance trait index (STTI): Salinity tolerance trait index (STTI) modified from dry matter or grain yield efficiency index suggested by Fageria (1992) to classify genotypes for tolerance to salinity. The formula used is as follows:  $STTI = (Y1/AY1) \times (Y2/AY2)$ . Where, Y1 = trait mean at low salinity level. AY1 = average trait of genotypes at low salinity level. Y2 = trait mean at high salinity level. AY2 = average trait of genotypes at high salinity level. 2. Salinity tolerance index (STI) was calculated as follows:  $STI = (STT1I + STT2I + \dots + STTnI)/n$ . Where, STT1I, STT2I ..... STTnI=Trait No.1, Trait No.2 ..... Trait No.n, and n = number of measured traits. When STI is > 1, it indicates that genotype is tolerant (T) to salinity. If STI is > 0.5 to 1, it indicates that genotype is moderately tolerant (MT) to salinity. If STI is > 0 to < 0.5, it indicates that genotype is sensitive (S) to salinity.

**Statistical analysis**

All data in each season were subjected to analysis of variance (ANOVA) of factorial experiment using MSTAT C21 and Assistat 7.7 software's, and comparisons of means were made using the least significant difference (LSD) test at P ≤ 0.05 and 0.01 levels of confidence, according to Snedecor and Cochran (1989). Moreover, each main plot was analyzed separately as a randomized complete block design (RCBD) and combined analysis of variance across seasons was computed after performing the homogeneity test.

**Results**

**Analysis of variance**

Combined analysis of variance across years (2012/13 and 2013/14) for eleven studied traits of 60 wheat genotypes (58 DH lines and two checks) that survived under all studied NaCl concentrations (out of a total of 121 genotypes) in the pots experiment using a factorial design based on randomized complete block arrangement is presented in Table (1). The main effects of genotypes, NaCl treatments and their interaction were significant (P ≤ 0.01) for all studied traits. The main effects of years (seasons) were significant (P ≤ 0.01) for six out of 11 studied traits, namely PH, NTPP, NSPP, GYPP, BYPP and HI. Mean squares due to genotypes x years (G x Y), genotypes x salinity levels (G x S) and genotypes x years x salinity levels (G x Y x S) interactions were significant (P ≤ 0.01) for all studied traits, suggesting that rank of genotypes was different from year to another, from one salinity treatment to another and from a combination of salinity level and year to another combination. For each salinity level, a separate analysis of variance was performed across seasons (years) on the actual number of genotypes survived and reached maturity (121 at 0 and 3000 ppm, 106 at 6000 ppm and 60 at 9000 ppm NaCl).

Table 1. Combined analysis variance of studied traits of genotypes survived under four NaCl levels in pots factorial experiment across seasons.

SOV	df	Sum of squares (SS) %										
		DTH		DTM		PH		NTPP		NSPP		NGPS
Years (Y)	1	0.09		0.10		6.38	**	57.36	**	54.11	**	0.02
Rep. / year	4	0.03		0.40		0.04		5.35		2.61		0.02
Salinity levels (S)	3	17.98	**	17.68	**	53.70	**	14.01	**	14.67	**	47.10
Y X S	3	0.19		0.10		2.99	**	8.93	**	11.26	**	0.62
Error (a)	12	1.16		0.32		0.52		0.77		8.21		0.30
Genotypes (G)	59	19.20	**	19.12	**	13.89	**	2.82	**	1.69	**	12.38
G X Y	59	9.52	**	9.87	**	4.57	**	1.99	**	1.33	**	8.67
G X S	177	33.33	**	33.53	**	8.29	**	2.99	**	1.93	**	18.64
G X Y X S	177	17.40	**	18.11	**	5.93	**	2.47	**	1.75	**	10.39
Error (b)	944	1.10		0.78		3.68		3.31		2.46		1.86
Total SS		1473219		1979969		360805		8493		18400		231822
		TGW		GYPP		SYPP		BYPP		HI		
Years (Y)	1	0.001		5.25	**	0.50		1.32	**	1.91	**	
Rep. / year	4	0.07		0.07		0.14		0.05		0.18		
Salinity levels (S)	3	43.73	**	41.76	**	57.14	**	57.21	**	18.16	**	
Y X S	3	0.28	**	4.33	**	1.33	**	1.88	**	0.97	**	
Error (a)	12	0.05		0.37		0.03		0.04		0.16		
Genotypes (G)	59	22.79	**	21.04	**	11.03	**	12.85	**	17.49	**	
G X Y	59	5.85	**	5.80	**	7.02	**	6.53	**	9.76	**	
G X S	177	17.47	**	12.90	**	13.42	**	11.79	**	31.77	**	
G X Y X S	177	9.01	**	8.25	**	8.81	**	7.92	**	19.38	**	
Error (b)	944	0.74		0.22		0.57		0.41		0.22		
Total SS		320270		22515		205867		332520		13058		

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

**Genotypic differences**

Means of the studied wheat genotypes exhibited a wide range, *i.e.* great difference between the maximum and minimum values under control as well as salinity stress treatments. The twelve highest and twelve lowest genotypes under all salinity levels for the studied phenological, agronomic and yield traits in the pots experiment are presented in Table (2).

In general, it was observed that increasing the salinity level caused a reduction (narrowness) in the range for most studied traits. Under 6000 and 9000 ppm NaCl salinity conditions, 15 and 61 genotypes, *i.e.* 12.4 and 50.4 %, respectively could not reach to maturity (not survived). Mean grain yield/plant of each genotype under each salinity treatment in pots experiment is presented in Table (3). The twelve highest genotypes (10% of 121 genotypes) in mean grain yield/plant, in descending order were the DH lines No. 109, 11, 94, 103, 79, 81, 106, 85, 9, 56, 84 and 14 under 9000 ppm, No. 93, 28, 109, 79, 8, 68, 14, 26, 94, 29, 9 and 11 under 6000 ppm, No. 93, 11, 8, 9, 78, 2, 94, 79, 85, 26, 24 and 92 under 3000 ppm and No. 33, 26, 49, 93, 77, 78, 9, 8, 48, 40, 28 and 11 under 0 ppm NaCl conditions. It is observed that the rank of genotypes for GYPP changed form one salinity to another. The genotypes L11 and L9 were amongst the 12 highest yielding genotypes under all salinity levels.

Table 2. List of wheat DH lines/cultivars showing the 12 highest means and range (between parenthesis) and 12 lowest means of studied traits under different NaCl concentration in pots experiment across seasons

NaCl ppm	conc.	Highest DH lines/cultivars	Range	Lowest DH lines/cultivars	Range
		Days to heading			
0		79, 78, 74, 102, 117, 101, 83, 116, 12, 9, 16, 69.	(83 - 79)	91, 58, 53, 24, 57, 86, 60, 49, 90, 66, 56, 50.	(66 - 64)
3000		78, 83, 79, 74, 101, 102, 112, 116, 117, 12, 88, 92.	(84 - 80)	86, 66, 21, 87, 59, 58, 57, 53, 48, 44, 40, 24.	(67 - 66)
6000		102, 9, 16, 78, 12, 79, 117, Sk.93, 27, Sd.1, 74, 83.	(90 - 84)	53, 18, 90, 37, 56, 35, 91, 87, 52, 44, 58, 48.	(71 - 68)
9000		78, 79, 16, 9, 83, 27, 81, 82, 117, 2, 11, 85.	(95 - 91)	18, 53, 94, 56, 21, 35, 87, 62, 57, 64, 32, 20.	(81 - 75)
		Days to maturity			
0		16, 12, 74, 102, 101, 117, 79, 78, 9, 27, 116, 22.	(113 - 111)	91, 53, 58, 90, 86, 24, 57, 35, 60, 37, 49, 66.	(97 - 93)
3000		116, 101, 102, 12, 16, 27, 74, 117, 11, 69, 83, 9.	(115 - 110)	91, 90, 86, 53, 35, 58, 57, 49, 48, 24, 66, 37.	(97 - 93)
6000		101, 9, 102, 16, 78, 12, 11, 79, 69, 27, 74, 83.	122 - 117)	34, 91, 111, 90, 20, 53, 104, 65, 18, 113, 86, 67.	(104 - 83)
9000		9, 16, 78, 83, 11, 79, 55, 2, 10, 13, 93, 27.	(131 - 124)	104, 65, 86, 67, 53, 113, 58, 29, 18, 97, 35, 111.	(114 - 108)
		Plant height (cm)			
0		9, 24, 33, L.25, 29, 37, 76, 84, 26, 28, 17, Sd.1.	(52.8 - 44.7)	65, 112, 6, 117, 114, 3, 96, 116, 113, 66, 51, 59.	(23.0 - 15.4)
3000		9, 24, 33, 15, 26, 22, 84, 17, 83, L.25, 8, 37.	(66.3 - 45.7)	65, 105, 114, 1, 101, 43, 112, 57, 60, 108, 69, 89.	(21.3 - 10.3)
6000		9, 33, 84, 93, 22, 76, 15, 5, 24, 45, 29, 73.	(37.9 - 27.1)	42, 105, 49, 41, 101, 65, 112, 117, 63, 96, 116, 113.	(7.8 - 3.5)
9000		9, 22, 84, Sk.8, 8, 13, 55, 26, 16, 17, 93, 21.	(23.0 - 14.7)	65, 19, 112, 117, 63, 96, 113, 28, 67, 97, 82, 57.	(5.9 - 3.8)
		No. of Tillers/spike			
0		2, 8, 9, 11, 24, 26, 33, 48, 49, 78, 79, 93.	(7.5 - 7.5)	113, 108, 74, 65, 62, 60, 55, 54, 51, 46, 45, 38.	(3.5 - 3.5)
3000		28, 109, 8, 26, 93, 68, 9, 11, 24, 48, 79, 94.	(7.0 - 6.3)	91, 66, 50, 41, 117, 115, 114, 112, 98, 42, 40, 114.	(3.0 - 2.6)
6000		11, 103, 109, 94, 104, 14, 93, 9, 24, 6, 31, 38.	(3.8 - 1.8)	79, 78, 48, 33, 26, 8, 2, 92, 91, 87, 85, 84.	(1.3 - 1.2)
9000		6, 51, 55, 60, 62, 65, 113, 3, 13, 16, 20, 21.	(1.8 - 1.5)	94, 93, 79, 78, 33, 26, 24, 11, 9, 8, 2, 109.	(1.3 - 1.2)
		No. of Spikes/plant			
0		2, 8, 9, 11, 24, 26, 33, 48, 49, 78, 79, 93.	(6.0 - 6.0)	113, 108, 74, 65, 62, 60, 55, 54, 51, 46, 45, 38.	(2.0 - 2.0)
3000		8, 26, 28, 93, 109, 9, 11, 24, 48, 68, 79, 94.	(5.5 - 5.0)	117, 115, 114, 113, 112, 108, 98, 91, 89, 74, 69, 66.	(2.0 - 2.0)
6000		11, 94, 103, 109, 14, 93, 104, 9, 24, 2, 3, 4.	(3.0 - 1.0)	117, 116, 113, 112, 111, 110, 107, 106, 102, 100, 99, 98.	(1.0 - 1.0)
9000		2, 3, 6, 8, 9, 10, 11, 13, 14, 15, 16, 17.	(1.0 - 1.0)	117, 113, 112, 111, 109, 107, 106, 104, 103, 97, 96, 94.	(1.0 - 1.0)
		No. of grains/spike			
0		33, 49, 56, 48, 77, 68, 26, 86, Sd.1, 78, 93, 85.	(54.4 - 40.0)	65, 31, 62, 113, 45, 16, 6, 74, 21, 60, 54, 23.	(18.4 - 11.7)
3000		33, 48, 49, L.25, Sk.93, 2, Sd.1, 85, 86, 77, Sk.8, 56.	(45.7 - 37.9)	31, 108, 45, 6, 65, 62, 54, 23, 113, 51, 64, 16.	(17.9 - 10.4)
6000		33, 56, 93, 2, 78, 83, 95, 74, 9, 80, 103, 77.	(37.4 - 25.7)	31, 113, 12, 7, 65, 60, 116, 67, 54, 25, 46, 69.	(8.3 - 3.8)
9000		84, 9, 8, 2, 10, 15, 17, 78, 80, Sk.8, 93, 70.	(28.7 - 16.0)	65, 25, 111, 85, 117, 58, 27, 92, 53, 81, 56, 109.	(6.2 - 4.9)
		1000 Grains weight (g)			
0		26, 102, 12, 91, 8, 14, 18, 101, 84, 83, 11, 49.	(49.0 - 39.5)	62, 46, 38, 45, 6, 54, 55, 74, 65, 31, 60, 51.	(18.7 - 5.8)
3000		8, 9, 78, 93, 2, 11, 94, 79, 85, 26, 24, 92.	(55.9 - 45.9)	62, 31, 65, 38, 108, 46, 6, 54, 45, 16, 113, 60.	(14.4 - 4.9)
6000		93, 79, 109, 70, 22, 8, 15, 71, 102, 87, 111, 74.	(40.2 - 35.5)	31, 65, 25, 60, 49, 46, 44, 7, 113, 38, 40, 73.	(8.2 - 1.3)
9000		9, 8, 80, 109, 2, 20, 11, 10, 94, 103, 21, 79.	(23.3 - 16.5)	Sk.93, 65, 25, 63, 58, 55, 32, 6, 59, 28, 57, 26.	(5.9 - 1.3)
		Grain yield/plant (g)			
0		33, 26, 49, 93, 77, 78, 9, 8, 48, 40, 28, 11.	(15.1 - 10.6)	62, 45, 65, 6, 46, 38, 31, 54, 74, 60, 51, 55.	(3.0 - 0.9)
3000		93, 11, 8, 9, 78, 2, 94, 79, 85, 26, 24, 92.	(15.9 - 11.4)	62, 31, 65, 38, 108, 46, 6, 54, 45, 16, 113, 60.	(3.6 - 1.2)
6000		93, 28, 109, 79, 8, 68, 14, 26, 94, 29, 9, 11.	(9.5 - 6.9)	31, 32, 38, 69, 46, 74, 25, 113, 40, 35, 6, 45.	(1.7 - 0.5)
9000		109, 11, 94, 103, 79, 81, 106, 85, 9, 56, 84, 14.	(7.5 - 4.1)	55, 6, 32, 5, 25, 18, 83, 65, 26, 17, 3, 16.	(1.2 - 0.6)
		Straw yield/plant (g)			
0		26, 33, 77, 49, 8, 9, 78, 17, 93, 100, 48, 84.	(50.9 - 39.7)	62, 31, 54, 45, 60, 46, 38, 108, 113, 6, 65, 74.	(16.4 - 7.3)
3000		77, 33, 48, 93, 40, 100, 109, 50, 49, 11, 68, 85.	(37.2 - 33.2)	54, 31, 108, 113, 69, 1, 43, 62, 101, 7, 61, 19.	(14.4 - 6.9)
6000		93, 85, 109, 94, 79, 8, 29, 17, 87, 14, 37, 26.	(33.3 - 24.9)	68, 69, 90, 96, 67, 63, 12, 7, 97, 113, 66, 54.	(4.0 - 0.7)
9000		84, 70, 33, 8, 97, 9, 17, 10, 93, 2, 20, 59.	(19.9 - 10.11)	5, 112, 53, 96, 92, 58, 82, 107, 113, 63, 56, 106.	(2.2 - 0.1)
		Biological yield/plant (g)			
0		33, 26, 49, 77, 8, 78, 9, 93, 17, 100, 48, 28.	(65.8 - 49.7)	62, 31, 45, 54, 46, 60, 38, 65, 6, 113, 108, 74.	(19.1 - 8.1)
3000		93, 11, 33, 48, 77, 109, 85, 78, 49, 94, 100, 50.	(51.7 - 44.2)	31, 54, 108, 62, 113, 69, 43, 7, 45, 1, 46, 61.	(18.4 - 8.9)
6000		93, 109, 94, 85, 79, 8, 29, 14, 17, 87, 26, L.25.	(42.8 - 30.6)	69, 66, 54, 61, 42, 89, 113, 62, 98, 63, 4, 7.	(5.9 - 1.7)
9000		84, 9, 8, 33, 70, 10, Sk.8, 97, 2, 20, 14, 93.	(23.9 - 12.3)	5, 96, 113, 63, 58, 77, 53, 104, 6, 67, 65, 107.	(4.4 - 0.7)
		Harvest index %			
0		24, 49, Sk.93, 33, 93, 26, 80, 40, 11, 48, 28, 81.	(23.7 - 21.3)	62, 65, 57, 6, 70, 59, 3, 51, 55, 25, 82, 74.	(14.1 - 10.4)
3000		2, 19, 1, 8, 24, 9, 26, 101, 10, 84, 28, 22.	(42.9 - 31.9)	62, 38, 40, 65, 46, 6, 32, 45, 55, 23, 60, Sd.1.	(16.1 - 9.5)
6000		68, 90, 96, 67, 12, 97, 69, 63, 11, 104, 7, 48.	(91.3 - 430)	32, 25, 31, 40, 74, 76, 38, 75, 33, 36, 46, 58.	(14.9 - 4.2)
9000		5, 112, 92, 53, 56, 82, 106, 81, 94, 109, 107, 11.	(87.7 - 61.7)	32, 17, 55, 3, 59, 26, Sk.8, 70, 25, 83, 78, 6.	(15.1 - 6.1)

Under the highest salinity level in this study (9000 ppm) some of 12 highest yielding (GYPP) DH lines were also amongst the 12 highest TGW (L9, L109, L11, L94, L103 and L79), NGPS (L84 and L9), NSPP (L9, L11 and L14), PH (L9), SYPP (L84 and L9), BYPP (L84, L9 and L14) and HI (L56, L81, L94, L109 and L11), but were amongst the 12 latest DTH (L79, L9, L81 and L11) and DTM (L9, L11 and L79) (Table 3). Under 6000 ppm NaCl some of the 12 highest GYPP DH lines showed also the highest BYPP (8 lines), SYPP (7 lines), TGW (4 lines), NSPP (6 lines) and NTPP (5 lines) and the latest in DTM and DTH (3 lines).

Ranks of the studied DH lines and checks for grain yield/plant under each salinity treatment and across the four treatments are presented in Table (4). Across the salinity stress and non- stress treatments, the highest 12 ranks were occupied by the DH lines L11, L8, L9, L94, L79, L109, L93, L85, L80, L103, L26 and L84, while the lowest 12 ranks were exhibited by DH lines L62, L31, L38, L54, L46, L108, L45, L6, L74, L113, L65 and L61. On the contrary, the lowest means of GYPP under 9000 ppm were observed for the DH lines L55, L6, L32, L5, L25, L18, L83, L65, L26, L17, L3 and L16 in ascending order (Tables 2 and 3).

Studied DH lines and checks that survived under 9000 ppm (60 genotypes) were grouped according to their grain yield/plant under the elevated level (efficient vs. non-efficient) as well as under the control (responsive vs. non-responsive) into four groups, i.e. efficient responsive (E-R), efficient non-responsive (E-NR), non-efficient responsive (NE-R) and non-efficient and non-responsive (NE-NR) genotypes for the elevated level 9000 ppm (Fig.1) and 6000 ppm (Fig.2). Grain yield/plant under 9000 ppm vs. 0 ppm NaCl (Fig.1) grouped the 60 genotypes into 16 efficient responsive genotypes, 9 efficient but not responsive, 12 non-efficient but responsive, and 23 non-efficient non-responsive.

Table 3. Mean grain yield/plant of the studied wheat genotypes under different salinity treatments in pots experiment across two years.

Geno- types	NaCl (ppm)				Geno- types	NaCl (ppm)			
	Control	3000	6000	9000		Control	3000	6000	9000
1	6.02	6.98	0	0	63	8.26	9.54	2.65	0
2	9.80	12.90	4.34	2.67	64	4.41	6.23	2.31	1.84
3	3.33	4.32	2.40	1.15	65	1.93	2.09	1.87	1.04
4	4.55	4.88	1.78	0	66	6.89	8.91	0	0
5	6.17	7.32	3.17	0.57	67	6.54	8.75	6.73	0
6	1.97	2.19	1.62	0.55	68	9.69	10.81	7.56	0
7	3.10	3.89	2.76	0	69	4.47	4.21	1.01	0
8	10.89	13.90	7.61	3.86	70	4.51	5.82	3.94	2.13
9	11.04	13.68	7.02	4.41	71	5.87	7.95	5.74	0
10	7.67	10.20	4.86	3.05	72	6.11	8.13	5.77	0
11	10.60	15.03	6.99	7.05	73	6.47	5.53	3.51	0
12	8.14	8.75	5.32	0	74	2.69	3.72	1.32	0
13	5.40	5.94	4.34	1.62	75	6.50	5.84	3.06	2.76
14	8.40	10.23	7.33	4.07	76	8.31	10.53	2.69	0
15	6.04	7.95	4.99	2.58	77	11.98	9.34	3.20	0
16	3.43	3.42	1.93	1.18	78	11.40	13.53	5.36	1.50
17	10.24	10.32	6.66	1.12	79	9.55	12.36	8.17	6.57
18	7.94	6.53	2.78	0.79	80	10.14	11.34	6.12	3.69
19	7.96	10.42	4.25	2.01	81	8.78	9.43	6.04	6.01
20	5.42	6.10	3.37	2.49	82	5.24	6.12	3.51	3.25
21	5.40	5.62	3.77	2.07	83	8.82	10.35	5.21	1.04
22	6.70	8.28	5.66	1.71	84	9.01	11.00	6.57	4.08
23	5.43	3.78	3.24	0	85	9.51	12.32	6.72	4.51
24	10.13	11.70	6.97	1.68	86	7.00	8.32	4.87	0
25	4.08	5.26	1.34	0.68	87	8.59	11.12	5.93	3.20
26	14.64	11.89	7.31	1.04	88	7.95	9.32	4.63	0
27	7.67	9.10	5.30	2.23	89	4.95	4.34	0	0
28	10.63	9.81	8.66	1.59	90	8.35	10.76	5.85	0
29	7.44	10.63	7.03	0	91	8.45	11.18	0	0
30	6.93	8.23	3.44	0	92	9.24	11.39	3.84	3.72
31	2.11	1.92	0.53	0	93	12.13	15.93	9.46	2.03
32	7.71	3.72	0.67	0.57	94	9.86	12.43	7.26	6.85
33	15.13	10.42	3.01	2.95	95	5.73	7.85	5.07	0
34	7.52	6.43	2.76	0	96	5.90	7.64	5.56	0
35	4.86	5.43	1.47	1.19	97	5.54	6.94	5.43	3.42
36	5.63	4.51	2.22	0	98	5.58	6.42	0	0
37	5.34	7.04	4.61	0	99	8.06	8.41	3.99	0
38	2.05	2.09	0.96	0	100	9.92	9.31	5.34	0
39	5.27	6.23	4.03	0	101	8.62	6.43	4.04	0
40	10.69	4.32	1.45	0	102	8.74	9.64	5.62	0
41	7.67	9.23	0	0	103	8.88	11.32	6.11	6.76
42	5.64	7.12	0	0	104	5.52	5.64	4.21	0
43	3.51	4.43	1.83	0	105	6.24	6.53	2.78	0

44	5.15	5.34	2.54	0	106	6.54	8.12	4.63	4.74
45	1.81	2.40	1.66	0	107	6.27	7.85	3.05	2.81
46	2.00	2.10	1.04	0	108	3.21	2.10	0	0
47	6.31	7.27	4.45	0	109	9.72	11.23	8.33	7.53
48	10.89	10.81	6.43	0	110	5.26	6.37	4.21	0
49	13.58	10.22	2.43	0	111	7.11	8.99	5.13	3.28
50	9.91	9.23	0	0	112	5.39	6.38	2.27	3.84
51	2.91	3.92	2.70	1.34	113	3.06	3.48	1.39	0
52	5.81	7.12	3.39	2.60	114	5.30	5.64	0	0
53	5.19	6.43	3.15	2.36	115	7.61	5.90	0	0
54	2.30	2.32	0	0	116	6.77	8.04	3.27	0
55	2.95	3.86	2.62	0.54	117	6.00	6.97	2.72	3.40
56	6.90	8.42	4.76	4.35	L. 25	6.11	7.64	0	0
57	3.82	4.94	3.93	2.32	Sd. 1	5.69	5.61	1.88	0
58	7.01	7.34	2.38	1.29	Sk. 8	4.64	5.45	3.73	1.69
59	4.13	5.93	4.27	1.31	Sk. 93	6.53	7.36	2.00	2.63
60	2.76	3.57	2.32	0	Mean	6.74	7.53	4.03	2.71
61	4.05	4.32	0	0	Max	15.13	15.93	9.46	7.53
62	0.84	1.23	0	0	Min	0.84	1.23	0.53	0.54

Table 4. Ranks of studied wheat genotypes for GYPP under each and across salinity treatments.

Geno- types	Rank at Control	3000	6000	9000	Combined	Geno- types	Rank at Control	3000	6000	9000	Combined
11	12	2	12	2	1	75	60	84	69	25	62
8	8	3	5	13	2	58	50	60	84	46	63
9	7	4	11	9	3	82	90	79	59	20	64
94	18	7	9	3	4	5	65	61	67	56	65
79	22	8	4	5	5	41	43	38	107	61	65
109	20	15	3	1	5	13	83	81	45	41	67
93	4	1	1	35	7	34	47	71	74	61	68
85	23	9	15	8	8	20	82	80	63	29	69
80	14	13	19	16	9	37	86	65	43	61	70
103	26	14	20	4	10	52	73	63	62	60	71
26	2	10	8	51	11	53	91	71	68	30	72
84	25	18	17	11	11	112	85	75	87	14	73
24	15	11	13	40	13	21	83	88	57	34	74
14	33	28	7	12	14	66	54	42	107	61	75
78	6	5	30	43	15	105	64	69	72	61	76
28	11	31	2	42	16	40	10	100	98	61	77
81	28	34	21	6	17	70	97	85	54	33	77
87	31	17	22	21	18	73	61	90	59	61	79
2	19	6	45	26	19	59	100	82	47	45	80
17	13	27	16	50	20	110	89	76	49	61	81
48	8	19	18	61	20	104	80	86	49	61	82
68	21	19	6	61	22	39	88	77	52	61	83
92	24	12	56	15	22	Sk. 8	95	91	58	39	84
33	1	24	71	23	24	57	103	95	55	31	85
10	43	30	39	22	25	L. 25	66	57	107	61	86
83	27	26	34	51	26	115	46	83	107	61	87
90	34	21	23	61	27	64	99	77	86	37	88
29	48	22	10	61	28	1	69	66	107	61	89
111	49	41	35	19	29	42	76	63	107	61	90
100	16	37	31	61	30	32	42	108	105	56	91
19	39	24	48	36	31	23	81	107	65	61	92
27	43	40	33	32	32	Sd. 1	75	89	91	61	93
56	53	45	40	10	32	98	78	74	107	61	94
102	29	32	27	61	34	36	77	97	88	61	95
106	57	51	41	7	35	44	92	93	81	61	96
77	5	35	66	61	36	35	94	92	97	47	97
22	56	48	26	38	37	51	111	104	77	44	98
12	37	43	32	61	38	3	106	100	83	49	99
49	3	29	82	61	39	114	87	86	107	61	100
67	57	43	14	61	39	4	96	96	94	61	101
88	40	36	41	61	41	7	108	105	74	61	102
15	68	53	37	28	42	25	101	94	100	55	103
97	79	68	29	17	43	16	105	112	90	48	104
76	35	23	78	61	44	55	110	106	80	59	104
86	51	47	38	61	44	43	104	98	93	61	106
99	38	46	53	61	46	89	93	99	107	61	107
72	66	50	24	61	47	69	98	103	103	61	108
63	36	33	79	61	48	60	112	110	85	61	109
71	72	53	25	61	49	61	102	100	107	61	110
107	63	55	70	24	50	65	119	118	92	51	111
101	30	71	51	61	51	113	109	111	99	61	111

91	32	16	107	61	52	74	113	108	101	61	113
96	71	57	28	61	53	6	118	115	96	58	114
30	52	49	61	61	54	45	120	113	95	61	115
50	17	38	107	61	54	108	107	116	107	61	116
95	74	55	36	61	56	46	117	116	102	61	117
47	62	62	44	61	57	54	114	114	107	61	118
117	70	67	76	18	58	38	116	118	104	61	119
116	55	52	64	61	59	31	115	120	106	61	120
Sk. 93	59	59	89	27	60	62	121	121	107	61	121
18	41	69	72	54	61						

The first group (E-R) that included lines 109, 11, 94, 103, 79, 81, 85, 9, 14, 84, 8, 90, 80, 87, 10 and 33 and second group (E-NR) that included lines 106, 56, 112, 97, 117, 111, 82, 107 and 75 could be recommended for direct use in Egyptian areas affected by high level of salinity (up to 9000 ppm NaCl), with giving priority to the first group that shows responsiveness, *i.e.* high GYPP if the salinity of the soil in these areas were ameliorated managed by the new technologies of salinity amendment. The third group (NE-R) that included lines L26, L93, L78, L28, L17, L24, L2, L83, L19, L18, L27 and L32 could be used only in non saline affected soils as high yielding genotypes in good soil.

Under 6000 ppm NaCl (Fig.2) the E-R group included the same DH lines of E-R group under 9000 ppm (Fig.2), except lines No. 33 and 92, but had eight more lines (No. 93, 28, 26, 24, 17, 78, 27 and 83). Also E-NR group under 6000 and 9000 ppm NaCl included four common DH lines (No. 56, 97, 106 and 111) (Fig.2). It is observed that under 6000 ppm NaCl, the DH line L93 followed by L28 were the best E-R lines, but were not amongst this group under 9000 ppm, suggesting the favorable interaction between these two genotypes and the salinity level 6000 ppm NaCl.

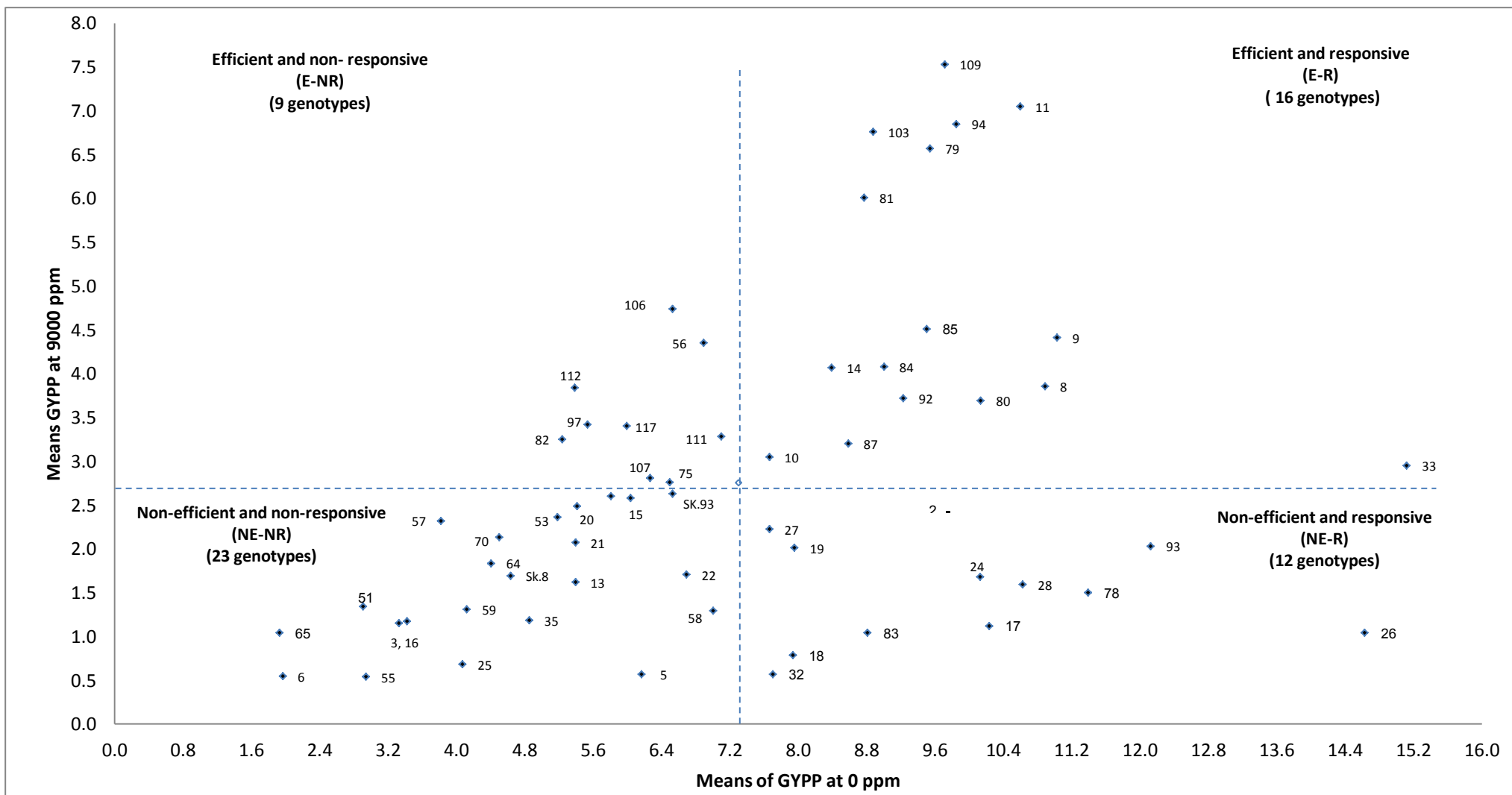


Figure 1. Relationships between means of grain yield/plant of 60 wheat genotypes under 0 ppm and 9000 ppm NaCl treatment in pot experiment across two seasons. Broken line repeated means of GYPP, (numbers from 1 to 60 refer to DH line number).



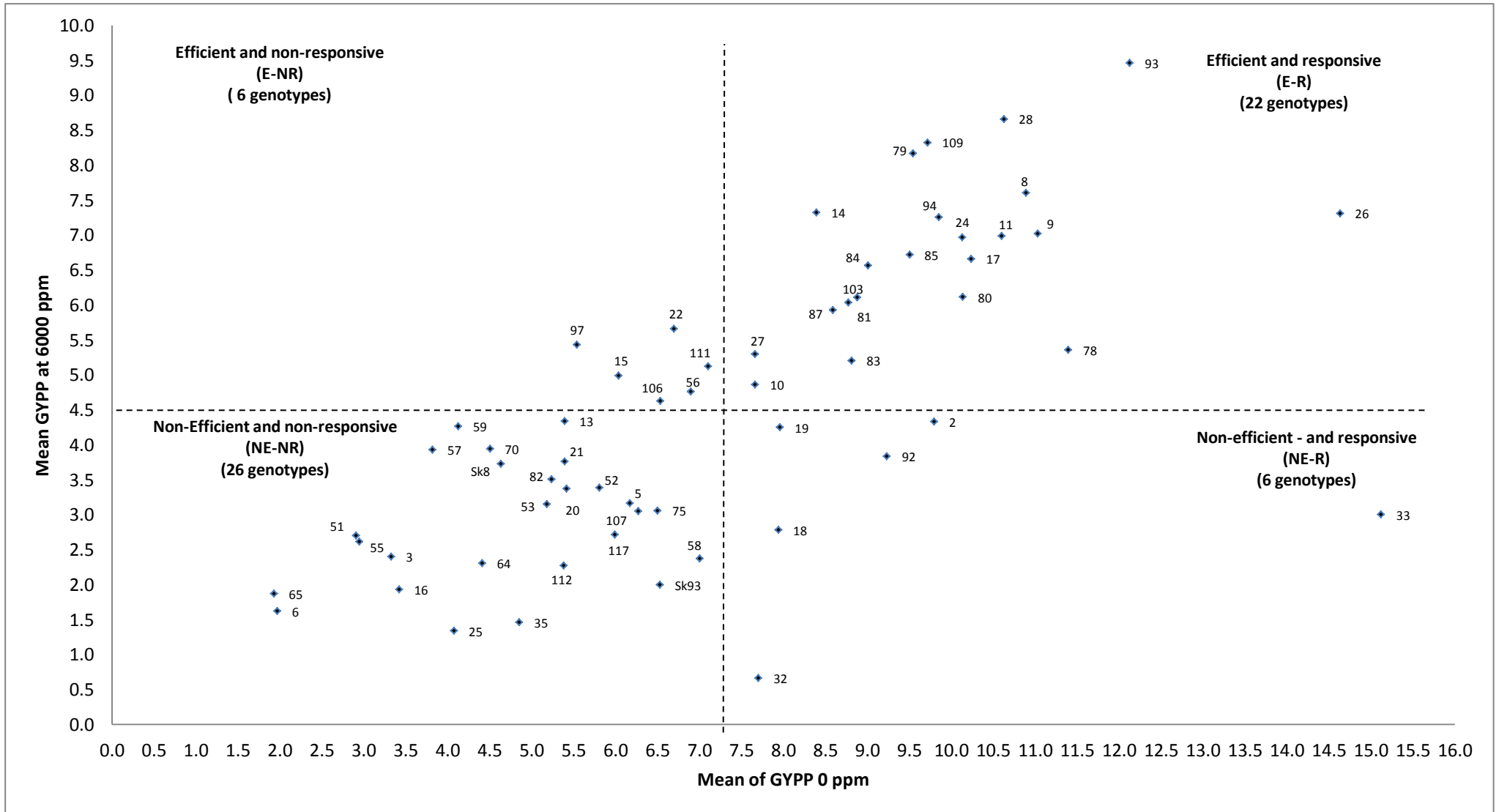


Figure 2. Relationships between means of grain yield/plant of 60 wheat genotypes at 0 ppm and 6000 ppm NaCl treatment in pot experiment across two seasons. Broken line repeated means of GYPP, (numbers from 1 to 60 refer to DH line number).

Table 5. Summary of means salinity tolerant trait indexes (STTI's) for ten of studied traits in pot experiment across seasons of 121 wheat genotypes evaluated under different NaCl stress levels.

NaCl ppm	Traits				
	DTH	DTM	PH	NTPP	NSPP
3000	1.006	1.006	1.069	1.031	1.073
6000	1.046	1.039	1.077	0.993	1.003
9000	1.035	1.015	1.088	0.998	1.030
NaCl ppm	Traits				
	NGPS	TGW	GYPP	SYPP	BYPP
3000	1.067	1.059	1.106	1.069	1.057
6000	1.096	1.037	1.127	1.099	1.076
9000	1.127	1.028	1.150	1.119	1.094

Table 6. Salinity tolerance index (STI) of studied DH lines/cultivars evaluated under 3000, 6000 and 9000 ppm NaCl stress and in pot experiment across seasons.

Geno- types	STI's			Geno- types	STI's			Geno- types	STI's		
	3000	6000	9000		3000	6000	9000		3000	6000	9000
1	0.703	0.000	0.000	43	0.398	0.482	0.000	85	1.795	1.751	1.247
2	1.628	1.432	1.750	44	0.787	0.649	0.000	86	1.262	0.846	0.000
3	0.494	0.600	0.774	45	0.386	0.537	0.000	87	1.497	1.651	1.055
4	0.701	0.443	0.000	46	0.324	0.349	0.000	88	1.409	1.368	0.000
5	1.027	1.028	0.036	47	1.016	1.266	0.000	89	0.574	0.267	0.000
6	0.290	0.360	0.372	48	2.000	1.193	0.000	90	1.503	0.839	0.000
7	0.524	0.419	0.000	49	2.007	0.705	0.000	91	1.226	0.471	0.000
8	2.035	2.084	2.320	50	1.370	0.000	0.000	92	1.536	1.172	0.975
9	2.182	2.270	2.598	51	0.376	0.500	0.563	93	2.351	2.856	1.768
10	1.361	1.407	1.682	52	0.873	0.983	0.000	94	1.868	2.367	1.608
11	2.051	1.756	1.727	53	0.759	0.830	0.613	95	1.018	1.257	0.000
12	1.223	0.732	0.000	54	0.322	0.240	0.000	96	0.933	0.622	0.000
13	0.916	0.786	0.956	55	0.559	0.616	0.586	97	0.749	0.607	0.936
14	1.454	1.802	1.439	56	1.365	1.485	1.031	98	0.808	0.339	0.000
15	1.080	1.273	1.328	57	0.611	0.892	0.611	99	1.328	1.201	0.000
16	0.520	0.660	0.717	58	1.068	1.070	0.635	100	1.597	1.357	0.000
17	1.808	1.913	1.593	59	0.709	0.874	0.839	101	1.001	0.665	0.000
18	1.104	1.042	0.884	60	0.357	0.306	0.000	102	1.429	1.473	0.000
19	1.303	0.732	0.749	61	0.597	0.299	0.000	103	1.521	1.942	1.526
20	0.721	0.828	1.121	62	0.265	0.227	0.000	104	0.739	0.761	0.000
21	0.746	1.033	1.119	63	1.188	0.586	0.000	105	0.801	0.420	0.000
22	1.234	1.483	1.280	64	0.680	0.701	0.925	106	1.081	1.180	0.924
23	0.623	0.385	0.000	65	0.254	0.249	0.294	107	0.960	0.958	0.756
24	1.832	1.854	1.358	66	0.887	0.347	0.000	108	0.287	0.000	0.000
25	0.635	0.722	0.561	67	1.075	0.736	0.000	109	1.805	2.410	1.632
26	2.305	2.230	1.504	68	1.770	0.996	0.000	110	0.841	1.039	0.000
27	1.308	1.466	0.963	69	0.459	0.294	0.000	111	1.150	1.273	0.886
28	1.609	1.506	0.729	70	0.772	1.039	1.184	112	0.705	0.507	0.671
29	1.585	1.808	0.000	71	0.933	1.096	0.000	113	0.322	0.272	0.000
30	1.148	1.082	0.000	72	1.031	1.157	0.000	114	0.571	0.000	0.000
31	0.272	0.307	0.000	73	0.912	0.924	0.000	115	0.963	0.000	0.000
32	0.912	0.992	0.970	74	0.384	0.611	0.000	116	0.998	0.745	0.000
33	2.433	2.129	2.146	75	0.927	1.145	0.937	117	0.759	0.609	0.764
34	0.972	0.521	0.000	76	1.368	1.428	0.000	Line 25	1.268	1.058	0.000
35	0.578	0.543	0.744	77	1.860	1.457	0.000	Sd. 1	1.037	0.697	0.000
36	0.672	0.682	0.000	78	2.008	1.824	1.538	Sk. 8	0.864	1.047	1.210
37	1.104	1.283	0.000	79	1.842	1.936	1.548	Sk. 93	0.927	0.676	0.792
38	0.428	0.534	0.000	80	1.641	1.393	1.563	Mean	1.079	1.022	1.113
39	0.803	0.953	0.000	81	1.344	1.407	1.263	Max	2.433	2.856	2.598
40	1.202	1.105	0.000	82	0.856	0.901	0.679	Min	0.254	0.084	0.036
41	1.126	0.414	0.000	83	1.622	1.575	1.217				
42	0.709	0.084	0.000	84	1.756	1.794	2.580				

0.000 = Not survived

**Salinity tolerance:**

Summarizing estimates of salinity tolerance trait indices (STTI's) of ten selected traits in Table (5) showed that under 6000 and 9000 ppm NaCl stress, tillers/plant (NTPP) followed by number of spikes/plant (NSPP) traits exhibited the lowest STTI mean estimate, while GYPP trait showed the highest estimates.

Salinity tolerance index (STI) estimates calculated as an average of eight selected traits (PH, NTPP, NSPP, NGPS, TGW, GYPP, SYPP and BYPP) in pots experiment (Table 6) showed that the 10 highest STI estimates under the three salinity stress treatments (3000, 6000 and 9000 ppm NaCl) were shown by L8, L9, L33, L84, L93, L10, L11, L2 and L109.. There are fourteen more genotypes (DH lines No. 14, 15, 17, 22, 24, 26, 56, 80, 81, 83, 85, 87, 92 and 103) that showed tolerance to salinity across the three salinity levels (3, 6 and 9 thousand ppm NaCl) expressed in STI estimate more than unity. On contrary, the most sensitive DH lines that exhibited 0 STI under both salinity stresses (6000 and 9000

ppm) were L 1, L 50, L 108, L 114 and L 115. There are fifteen more genotypes (DH lines No. 23, 31, 42, 46, 54, 60, 61, 62, 65, 66, 89, 91, 98, 105 and 113) showing sensitivity to salinity at both 6000 and 9000 ppm salinity treatments expressed in  $STI < 0.5$ .

Based on STI's calculated across eight traits at measured in the pots experiment, the 121 wheat genotypes were grouped into four categories at 9000 ppm NaCl level, namely tolerant (29 genotypes), moderately tolerant (30 genotypes), sensitive (11 genotypes) and very sensitive (not survived) (51 genotypes) (Table 9). Number of tolerant, moderately tolerant, sensitive and very sensitive genotypes was 54, 40, 22 and 5 under 6000 ppm and 61, 44, 16 and 0 under 3000 ppm Na Cl conditions, respectively.

It is worthy to note that under 9000 ppm NaCl, only Sakha 8 was tolerant but ranked in the 24<sup>th</sup> place and Sakha 93 cultivar was moderately tolerant (MT) and ranked in the 13<sup>th</sup> place in this MT category, while Sids 1 and Line 25 were very sensitive (did not survive). Under 6000 ppm NaCl, the Egyptian cultivar Sakha 8 and the promising line (Line-25) were tolerant, while Sids 1 and Sakha 93 were moderately tolerant.

The 60 survived genotypes at all stress treatments were categorized on the bases of their mean grain yield/plant trait and STTI estimates of the same trait into six groups, *i.e.* high yielding – tolerant (HY-T) high yielding - moderately tolerant (HY-MT), high yielding – sensitive (HY-S), low yielding – tolerant (LY-T), low yielding – moderately tolerant (LY-MT) and low yielding – sensitive (LY-S) at 9000 ppm (Fig. 5) and at 6000 ppm (Fig. 6).

The six groups HY-T, HY-MT, HY-S, LY-T, LY-MT and LY-S included 21, 4, 13 and 2, 14 and 19 genotypes, under 9000 ppm (Fig. 5) and 30, 2, 0, 2, 14, 12 genotypes under 6000 ppm NaCl (Fig. 5) The first group included lines No. 109, 11, 94, 79, 103, 81, 9, 85, 8, 84, 106, 56, 14, 111, 87 and 80 under both 6000 and 9000 ppm, NaCl Lines 109 and 93 were the best HY-T genotype under 9000 and 6000 ppm NaCl, respectively. It is worthy to note that no high-yielding sensitive line was observed under both 6000 and 9000 ppm NaCl.

Table 7. Salt tolerant categories of 117 DH Lines and 4 cheeks based on Salt tolerance index (STI) for eight selected traits under 3000, 6000 and 9000 ppm NaCl stress conditions.

Salt tolerance category	Salt tolerance index (STI)	No. of genotypes	DH lines/cultivars	STI	
				Mean	Range
3000 ppm					
Tolerant	STI > 1	61	33, 93, 26, 9, 11, 8, 78, 49, 48, 94, 77, 79, 24, 17, 109, 85, 68, 84, 80, 2, 83, 28, 100, 29, 92, 103, 90, 87, 14, 102, 88, 50, 76, 56, 10, 81, 99, 27, 19, L. 25, 86, 22, 91, 12, 40, 63, 111, 30, 41, 37, 18, 106, 15, 67, 58, Sd.1, 72, 5, 95, 47, 101.	1.494	(2.43 - 1.01)
Moderately Tolerant	STI (>0.5 - 1)	44	116, 34, 115, 107, 96, 71, Sk.93, 75, 13, 32, 73, 66, 52, Sk.8, 82, 110, 98, 39, 105, 44, 70, 53, 117, 97, 21, 104, 20, 59, 42, 112, 1, 4, 64, 36, 25, 23, 57, 61, 35, 89, 114, 55, 7, 16.	0.768	(0.99 - 0.52)
Sensitive	STI (< 0.5)	16	3, 69, 38, 43, 45, 74, 51, 60, 46, 113, 54, 6, 108, 31, 62, 65.	0.351	(0.49 - 0.25)
6000 ppm					
Tolerant	STI > 1	54	93, 109, 94, 9, 26, 33, 8, 103, 79, 17, 24, 78, 29, 14, 84, 11, 85, 87, 83, 28, 56, 22, 102, 27, 77, 2, 76, 10, 81, 80, 88, 100, 37, 15, 111, 47, 95, 99, 48, 106, 92, 72, 75, 40, 71, 30, 58, L.25, Sk.8, 18, 110, 70, 21, 5.	1.512	(2.86 - 1.03)
Moderately Tolerant	STI (>0.5 - 1)	40	68, 32, 52, 107, 39, 73, 82, 57, 59, 86, 90, 53, 20, 13, 104, 116, 67, 12, 19, 25, 49, 64, Sd.1, 36, Sk.93, 101, 16, 44, 96, 55, 74, 117, 97, 3, 63, 35, 45, 38, 34, 112.	0.734	(0.99 - 0.51)
Sensitive	STI (< 0.5)	22	51, 43, 91, 4, 105, 7, 41, 23, 6, 46, 66, 98, 31, 60, 61, 69, 113, 89, 65, 54, 62, 42.	0.339	(0.50 - 0.08)
Very sensitive	STI = 0	5	1, 50, 108, 114, 115.	0	—
9000 ppm					
Tolerant	STI > 1	29	9, 84, 8, 33, 93, 2, 11, 10, 109, 94, 17, 80, 79, 78, 103, 26, 14, 24, 15, 22, 81, 85, 83, Sk.8, 70, 20, 21, 87, 56.	1.549	(2.59 - 1.03)
Moderately Tolerant	STI (>0.5 - 1)	30	92, 32, 27, 13, 75, 97, 64, 106, 111, 18, 59, 29, Sk.93, 3, 117, 107, 19, 35, 28, 16, 82, 112, 58, 53, 57, 86, 55, 77, 51, 25.	0.77	(0.98 - 0.56)
Sensitive	STI (< 0.5)	11	67, 6, 104, 52, 96, 63, 65, 60, 62, 113, 5,	0.294	(0.47 - 0.04)
Very sensitive	STI = 0	51	1, 4, 7, 12, 23, 30, 31, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 54, 61, 66, 68, 69, 71, 72, 73, 74, 76, 88, 90, 91, 95, 98, 99, 100, 101, 102, 105, 108, 110, 114, 115, 116, L.25, Sd.1.	0	—

**Superiority of HY-T over LY-S genotypes:**

To describe the differences between (HY-T) and (LY-S) groups of genotypes data of selected characters were averaged for the three highest (HY-T) genotypes and the three lowest (LY-S) genotypes at 9000 ppm NaCl stress level and presented in Table (8). These genotypes were L109, L11 and L94 for HY-T group and L5, L6 and L65 for LY-S group.

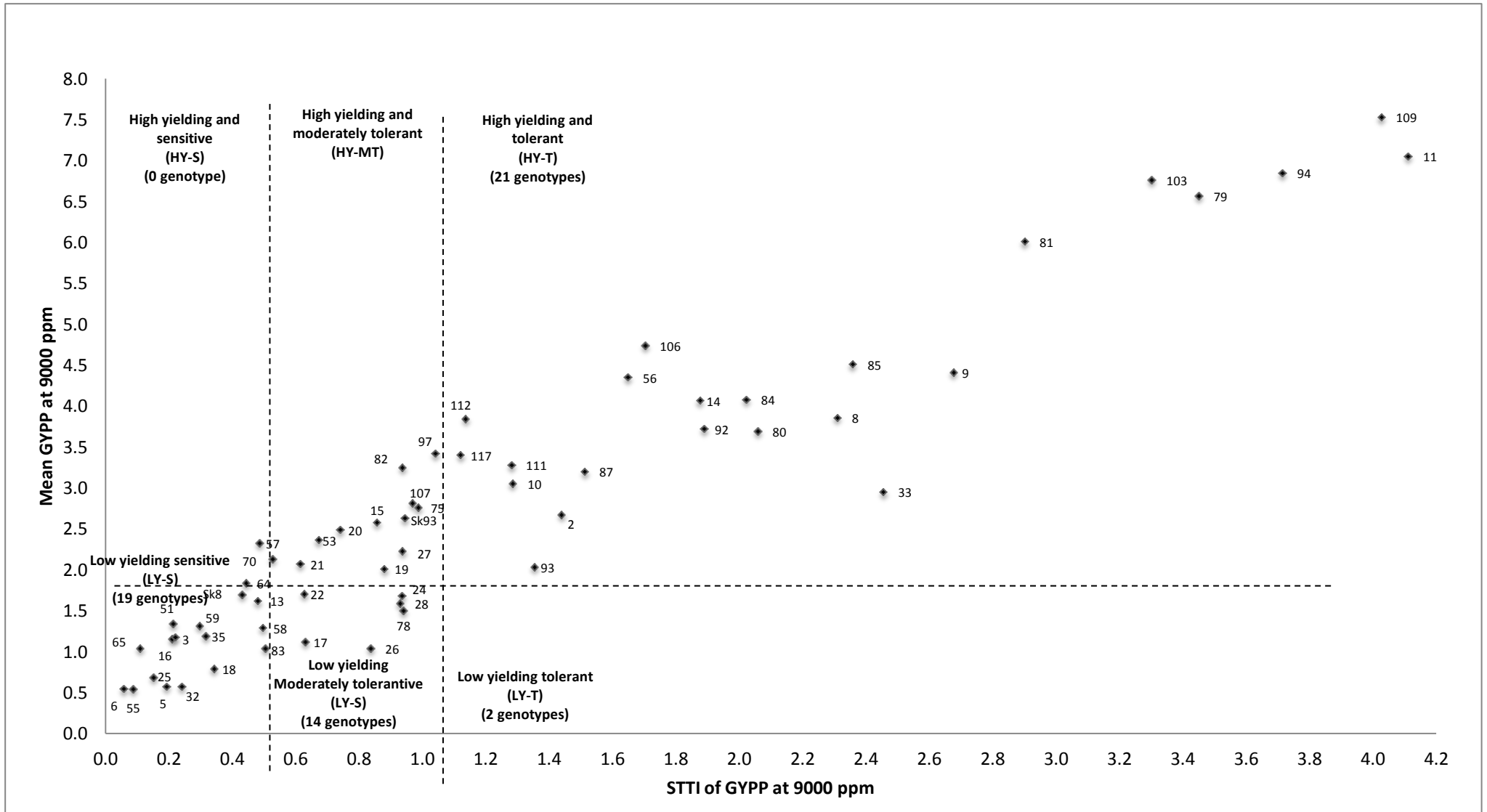


Figure 5. Relationships between mean grain yield/plant of 60 wheat genotypes and their STTI's under 9000 ppm NaCl treatment in pot experiment across two seasons. Broken line repeated mean of GYPP and its STTI, (numbers from 1 to 60 refer to DH line number).

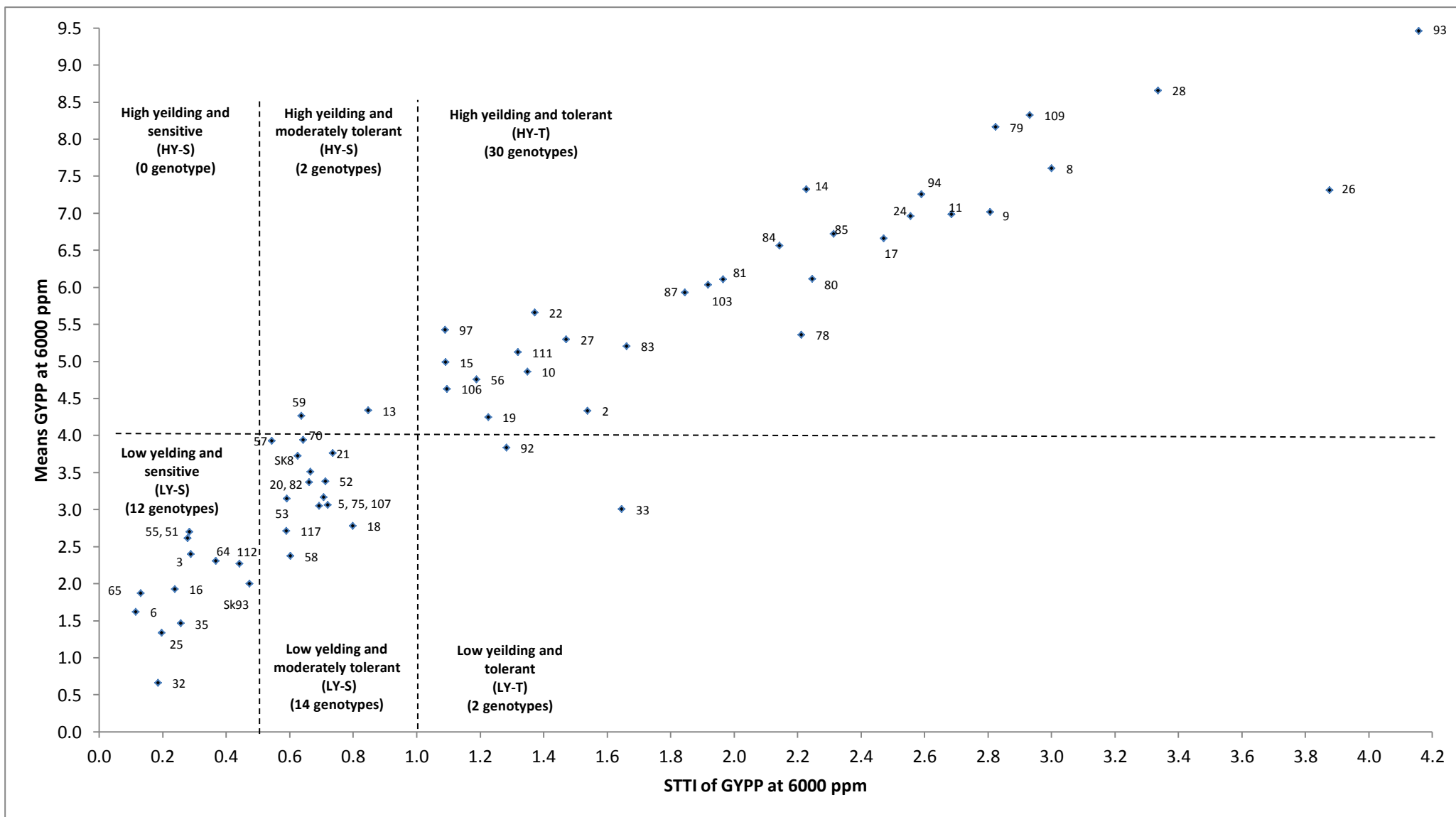


Figure 6. Relationships between mean grain yield/plant of 60 wheat genotypes and their STTI's under 6000 ppm NaCl treatment in pot experiment across two seasons. Broken line repeated mean of GYPP and its STTI, (numbers from 1 to 60 refer to DH line number).

Table 8. Superiority (%) of high-yielding (HY-T) over low-yielding sensitive (LY-S) DH lines under 9000 ppm evaluated under different NaCl levels (0, 3000, 6000 and 9000 ppm) for selected traits.

Traits	T	S	Superiority %	T	S	Superiority %
	Control			3000		
PH	36.92	22.66	38.6**	34.13	25.47	25.4**
NTPP	7.33	4.17	43.1**	6.50	3.48	46.5**
NGPS	33.66	20.38	39.5**	32.87	20.52	37.6**
TGW	37.28	20.66	44.6**	48.58	15.56	68.0**
GYPP	10.06	3.36	66.6**	12.90	3.87	70.0**
SYPP	37.50	19.57	47.8**	33.88	18.71	44.8**
BYPP	47.56	22.93	51.8**	46.77	22.58	51.7**
HI	21.12	13.79	34.7**	27.51	15.67	43.0**
	6000			9000		
PH	19.56	15.47	20.9**	8.63	4.14	52.0**
NTPP	3.35	1.63	51.3**	1.26	1.17	7.1*
NGPS	20.48	12.07	41.1**	11.99	9.16	23.6**
TGW	28.63	12.87	55.0**	17.95	8.21	54.3**
GYPP	7.52	2.22	70.5**	7.14	0.72	89.9**
SYPP	22.67	8.87	60.9**	4.03	2.13	47.1**
BYPP	30.19	11.09	63.3**	11.18	2.85	74.5**
HI	29.57	21.25	28.1**	64.01	42.36	33.8**

\* and \*\* indicate significance at 0.05 and 0.01 probability levels, respectively.

Under 9000 ppm NaCl conditions, significant higher values were exhibited in HY-T than in Ly-S by 89.9% for GYPP followed by 74.5% for BYPP, 54.3% for TGW, 52.0% for PH, 47.1% for SYPP, 33.8% for HI, 30.9% for NGPS and 7.7% for NTPP. The same trend of superiority of HY-T over LY-S lines was observed under 6000, 3000, and 0 ppm NaCl levels in a little less magnitude for GYPP, BYPP, TGW and PH and in much higher magnitude for NTPP trait. Regarding to DTH and DTM (data not presented in Table 8), it is worthy to note that though DTH and DTM of HY-T lines increased (delayed) by increasing salinity, reaching to 12 and 16 days, respectively at 9000 ppm as compared to 0 ppm NaCl, these two traits of LY-S lines showed a decrease (earliness), that reached 13 and 25 days, respectively.

#### Characterization of the ten best DH lines:

Most important characters of the best ten lines (highest yielding under 9000 ppm NaCl and under 6000 ppm NaCl in this study six are summarized and presented in Table (9). These lines are characterized as follows:

- L 109:** This DH line is tolerant to salinity at 6000 and 9000 ppm NaCl, responsive to good environment and the highest yielding of grains under 9000 ppm salinity level (Fig. 7) It showed superiority in grain yield over the best check (Sakha 93) at this salinity by 186.3 %. It was of moderate salinity tolerance at germination and seedling stages under 9000 ppm NaCl (Table9).
- L 11:** This DH line is tolerant at 6000 and 9000 ppm NaCl and the second highest in GYPP at 9000 ppm NaCl (Fig. 8). Its superiority in GYPP over Sakha 93 was 168.1 %. It was tolerant to salinity at germination and seedling stages under 9000 ppm NaCl conditions (Table 9).
- L 94:** This DH line is tolerant to salinity at 6000 and 9000 ppm NaCl, responsive to the improved environment and the 3<sup>rd</sup> highest yielding under 9000 ppm salinity level. Its superiority over Sakha 93 in GYPP at 9000 ppm was 160.5 %. It was also tolerant at germination and seedling stages under 9000 ppm NaCl level (Table 9).

Table 9. Salinity tolerance and superiority of the highest yielding DH lines at 9000 and 6000 ppm NaCl.

Ser. No.	DH line	GYPP (g) at salinity of		Superiority% over the best check	Tolerance (T) at reproduct-ive stage		Responsiveness	*Tolerance at Germination & seedling at 9000
		9000 ppm	6000 ppm		9000 ppm	6000 ppm		
1	L109	7.53		186.3	T	T	R	MT
2	L11	7.05		168.1	T	T	R	T
3	L94	6.85		160.5	T	T	R	T
4	L103	6.76		157.0	T	T	R	T
5	L79	6.57		149.8	T	T	NR	T
6	L81	6.01		128.5	T	T	NR	MT
	Sakha 93	2.73		----				
7	L93		9.46	152.9	T	T	R	T
8	L28		8.66	131.6	MT	T	R	S
9	L26		7.31	95.5	T	T	R	S
10	L24		6.97	86.4	T	T	R	T
	Sakha 8		3.74	----				

R= Responsive, NR= Non- responsive, \*( Al-Naggar *et al.*, 2015).



Figure 7. Plants of the DH line L109 at harvest under 0, 3000, 6000 and 9000 ppm NaCl.

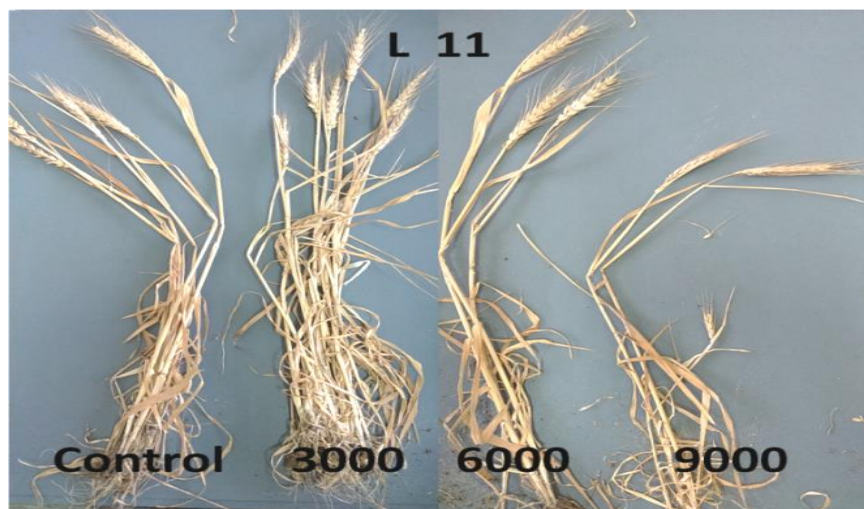


Figure 8. Plants of the DH line L11 at harvest under 0, 3000, 6000 and 9000 ppm NaCl.



Figure 9. Plants of the DH line L93 at harvest under 0, 3000, 6000 and 9000 ppm NaCl.

- L 103:** This line showed the 4<sup>th</sup> highest GYPP under 9000 ppm NaCl (157% superiority over Sakha 93), tolerant at both 9000 and 6000 ppm NaCl, responsive and tolerant at and seedling germination stages (Table 9).
- L 79:** It is tolerant to salinity at 6000 and 9000 ppm, but non-responsive to the non-stressed environment (0 ppm NaCl). It ranks the 5<sup>th</sup> in GYPP under 9000 ppm and is superior over the best check by 149.8 %. It is tolerant to salinity at 9000 ppm NaCl at germination and seedling stages (Table 9).

- L 81:** It is tolerant to salinity at 6000 and 9000 ppm, but non-responsive. It ranks the 6<sup>th</sup> for GYPP under 9000 ppm and showed superiority over the best check by 128.5 %. However, it is moderately tolerant at germination and seedling stages at 9000 ppm NaCl level (Table 9).
- L 93:** This line showed the highest GYPP under 6000 ppm NaCl level and showed a superiority of 152.9 % over the best check under this level of salinity (Sakha 8). It is tolerant at both levels of salinity (6000 and 9000 ppm) NaCl at reproductive stage (Fig. 9) as well as at germination and seedling stages (Table 9).
- L 28:** This line showed the second highest GYPP (131.6 % superiority over Sakha 8) under 6000 ppm NaCl and was moderately tolerant at reproductive stage under 6000 and tolerant at 9000 ppm NaCl and was responsive to control (non-stress) treatment. But it showed sensitivity to 9000 ppm NaCl at germination and seedling stages (Table 9).
- L 26:** This line ranked the 3<sup>rd</sup> in GYPP under 6000 ppm NaCl and was at tolerant at reproductive stage under both salinity levels (6000 and 9000 ppm NaCl). It showed superiority over the best check (Sakha 8) at 6000 ppm NaCl levels by 95.5% and was responsive to the improved (non-stressed) environment. However, it showed the 4<sup>th</sup> highest GYPP under 6000 ppm NaCl level at germination and seedling stages (Table 9).
- L 24:** This line showed the 4<sup>th</sup> highest GYPP under 6000 ppm NaCl (84.4 % superiority over Sakha 8). It showed tolerance at germination, seedling and reproductive stages at 6000 and 9000 ppm NaCl levels (Table 9).

## Discussion

Screening large numbers of genotypes in the field is notoriously difficult because of the variability of salinity within fields (Daniells *et al.*, 2001) and the enormous potential for interaction with other environmental factors, ranging from soil chemical and physical properties to temperature, light flux density and seasonal fluctuation in rainfall. Screening technique has, therefore, often been used under controlled conditions. Consequently, prediction of field performance is commonly carried out in trial plots method where the salinity of the medium can be readily adjusted to required values (Francios and Mass, 1994). Large numbers of bread wheat genotypes have been screened for salt tolerance in greenhouse; the criteria being biomass production at high salinity (up to 25 mM NaCl) relative to biomass in control conditions (Kingsbury and Epstein, 1984), and a screen by Sayed (1985) of 5000 wheat lines under solution culture, based on survival at high salinity, showed considerable genetic diversity amongst tested genotypes and lines.

The present study was conducted in pots under controlled salinity conditions to screen 117 DH lines produced from the cross between the salt tolerant Egyptian cultivar Sakha-8 and the promising high yielding line (Line-25) *via* anther culture technique (Doghma, 2007) for their salinity tolerance along with their parents and two check cultivars. The main effects of genotypes, NaCl treatments and their interaction were significant ( $P \leq 0.01$ ) for all studied traits. The main effects of years (seasons) were significant ( $P \leq 0.01$ ) for six out of 11 studied traits, namely PH, NTPP, NSPP, GYPP, BYPP and HI, indicating that environmental conditions prevailed in the two seasons (weather and/or soil conditions) were different to the extent that affected on such traits. The soil used in the 1<sup>st</sup> season was sand, while it was a mixture of 1 sand : 1 peat moss (v/v) in the second season. The most affected traits by sand soil in the 1<sup>st</sup> season were NTPP and NSPP (where both numbers were very low) and consequently affected on GYPP, BYPP and HI traits. Significance of main effects of wheat genotypes, salinity treatments and seasons of testing and their interactions of the present study confirm the findings of previous investigators (Munir *et al.*, 2013 and Al-Naggar *et al.*, 2015). Moreover, significant interaction between genotypes and salinity levels indicates that selection is possible to be practiced under a specific salinity treatment.

It was observed that salinity treatment effects were more pronounced than genotypes effects on all studied traits, except DTM and DTH, where the genotypes effects were more pronounced than salinity treatment effects (Table 1). This was expressed *via* the percentage of sum squares for each component to the total sum of squares, which indicated that salinity levels contributed higher percentage to the total variance than that reported by other investigators (Munir *et al.*, 2013). The highest contribution to total variance in this experiment was shown by salinity levels in six traits (PH, NGPS, TGW, GYPP, SYPP and BYPP) followed by years for two traits (NTPP and NSPP) and G x S for three traits (DTH, DTM and HI). Mean squares due to genotypes at all studied salinity treatments were significant ( $P \leq 0.01$ ), suggesting the existence of significant differences among survived genotypes under respective salinity levels for all studied characters. Such genotypic differences in studied traits under no salinity as well as elevated levels of salinity were also recorded by previous investigators in wheat (Khan *et al.* 2012, Asgaris *et al.* 2012 and Al-Naggar *et al.*, 2015).

Under 6000 and 9000 ppm NaCl salinity conditions, 15 and 61 genotypes, *i.e.* 12.4 and 50.4 %, respectively could not reach to maturity (not survived). The twelve highest genotypes (10% of 121 genotypes) in mean grain yield/plant, in descending order were the DH lines No. 109, 11, 94, 103, 79, 81, 106, 85, 9, 56, 84 and 14 under 9000 ppm, No. 93, 28, 109, 79, 8, 68, 14, 26, 94, 29, 9 and 11 under 6000 ppm, No. 93, 11, 8, 9, 78, 2, 94, 79, 85, 26, 24 and 92 under 3000 ppm and No. 33, 26, 49, 93, 77, 78, 9, 8, 48, 40, 28 and 11 under 0 ppm NaCl conditions. It is observed that the rank of genotypes for GYPP changed form one salinity to another. The genotypes L11 and L9 were amongst the 12 highest yielding genotypes under all salinity stress and non-stress conditions. For GYPP the DH line L109 ranked 1<sup>st</sup> under 9000 ppm and 3<sup>rd</sup> under 6000 ppm, while L11 ranked 2<sup>nd</sup> under 9000 ppm, 12<sup>th</sup> under 6000 ppm, 2<sup>nd</sup> under 3000 ppm and 12<sup>th</sup> under 0 ppm NaCl conditions. It is worthy to note that the five DH lines L11, L94, L79, L85 and L9 were amongst the twelve highest yielding genotypes under all elevated levels of salinity (3000, 6000 and 9000 ppm NaCl). The grain yield/plant of the best check was 2.63g for Sakha 93 under 9000 ppm, 3.73g for Sakha 8 under 6000 ppm, 7.64g for



Line25 under 3000 ppm and 6.53g for Sakha 93 under 0 ppm, so the best DH line showed higher GYPP by 186.3% for L109 under 9000 ppm, 153.6% for L93 under 6000 ppm, 108.5% for L93 under 3000 ppm and 131.7% for L33 under control (0 ppm salinity level) over the best respective check. Results showed that under 6000 and 9000 ppm NaCl stress, tillers/plant (NTPP) followed by number of spikes/plant (NSPP) traits exhibited the lowest STTI mean estimate, while GYPP trait showed the highest estimates, indicating that tillering (vegetative) stage is more sensitive to salinity than maturity stage. Many investigators also reported a similar conclusion (e.g. El-Hendawy *et al.*, 2005). The present results indicated that grain yield trait was the most tolerant one to the severe (9000 ppm NaCl) and medium (6000 ppm NaCl) salinity stress in this study.

Under 9000 ppm NaCl (Fig.1), the efficient-responsive group (E-R) that included lines 109, 11, 94, 103, 79, 81, 85, 9, 14, 84, 8, 90, 80, 87, 10 and 33 and the efficient non-responsive group (E-NR) that included lines 106, 56, 112, 97, 117, 111, 82, 107 and 75 could be recommended for direct use in Egyptian areas affected by high level of salinity (up to 9000 ppm NaCl), with giving priority to the first group that shows responsiveness, *i.e.* high GYPP if the salinity of the soil in these areas were ameliorated managed by the new technologies of salinity amendment. The third group (NE-R) that included lines L26, L93, L78, L28, L17, L24, L2, L83, L19, L18, L27 and L32 could be used only in non saline affected soils as high yielding genotypes in good soil. Under 6000 ppm NaCl (Fig.2) the E-R group included the same DH lines of E-R group under 9000 ppm (Fig.1), except lines No. 33 and 92, but had eight more lines (No. 93, 28, 26, 24, 17, 78, 27 and 83). Also E-NR group under 6000 and 9000 ppm NaCl included four common DH lines (No. 56, 97, 106 and 111) (Fig.2). It is observed that under 6000 ppm NaCl, the DH line L93 followed by L28 were the best E-R lines, but were not amongst this group under 9000 ppm, suggesting the favorable interaction between these two genotypes and the salinity level 6000 ppm NaCl.

Data showed that the 10 highest STI estimates under the three salinity stress treatments (3000, 6000 and 9000 ppm NaCl) were shown by L8, L9, L33, L84, L93, L10, L11, L2 and L109, indicating that these DH lines are the most tolerant genotypes, in descending order, in this study under the three studied elevated salinity treatments 3000, 6000 and 9000 ppm NaCl. There are fourteen more genotypes (DH lines No. 14, 15, 17, 22, 24, 26, 56, 80, 81, 83, 85, 87, 92 and 103) that showed tolerance to salinity across the three salinity levels (3, 6 and 9 thousand ppm NaCl) expressed in STI estimate more than unity. The relative ranking of genotypes varied between the different treatments, which may be due to the difference in interactions between genotype and salinity stress because of the complex nature of salt tolerance mechanisms (Khan *et al.*, 2010 and Al-Naggar *et al.*, 2015), However, the salt tolerance response of some genotypes (24) was stable across the different NaCl concentrations.

Under 9000 ppm NaCl conditions, significant higher values were exhibited in high-yielding tolerant (HY-T) than in low-yielding sensitive (LY-S) DH lines by 89.9% for GYPP followed by 74.5% for BYPP, 54.3% for TGW, 52.0% for PH, 47.1% for SYPP, 33.8% for HI, 30.9% for NGPS and 7.7% for NTPP. The same trend of superiority of HY-T over LY-S lines was observed under 6000, 3000, and 0 ppm NaCl levels in a little less magnitude for GYPP, BYPP, TGW and PH and in much higher magnitude for NTPP trait. Regarding to DTH and DTM, it is worthy to note that though DTH and DTM of HY-T lines increased (delayed) by increasing salinity, reaching to 12 and 16 days, respectively at 9000 ppm as compared to 0 ppm NaCl, these two traits of LY-S lines showed a decrease (earliness), that reached 13 and 25 days, respectively. Such differential response of HY-T and LY-S lines to DTH and DTM affected on their grain filling period (GFP), which was estimated as the period between flowering and physiological maturity. The estimated GFP increased in favor of HY-T lines by increasing salinity level; such increase of HY-T over LY-S reached 16 days (80.0%) at 9000 ppm NaCl. This means that LY-S lines had shorter GFP than HY-T lines at the highest salinity levels in this experiment, which might be considered one of reasons of lower GYPP for LY-S than HY-T lines.

In wheat, salt tolerance is associated with low rates of transport of  $\text{Na}^+$  to shoots with high selectivity for  $\text{K}^+$  over  $\text{Na}^+$  (Gorham *et al.*, 1987, 1990). Tolerant genotypes of bread wheat have a low rate of  $\text{Na}^+$  accumulation and enhanced  $\text{K}^+/\text{Na}^+$  discrimination, a character controlled by a locus (Knal) on chromosome 4D (Dubcovsky *et al.*, 1996). The gene or genes associated with this locus have not been identified (Munns *et al.*, 2006). In addition, genotype tolerance to high  $\text{Na}^+$  concentrations in leaves may differ, assumedly due to differences in compartmentation efficiency in leaf vacuoles; this trait has been characterized as "tissue tolerance" (Munns, 2005 and Flowers, 2004).

Hybridization between a salt tolerant genotype (Sakha 8) and a promising high-yielding genotype (Line 25) followed by producing doubled haploid lines *via* anther culture technique had therefore been successful in developing transgressive segregants of superior grain yield than both parents under all salt stress and non stress conditions in the present investigation. This technique besides its advantage in shortening the time (5 to 6 generations of selfing) required for reaching complete homozygosity of the pure (inbred) lines, it proved a great success in developing improved and perfect homozygous genotypes in many countries such as China, France, Hungary and Canada (Hu *et al.*, 1983, DeBuyser *et al.*, 1987 and DePaww *et al.*, 2011). Transgressive segregation, a well known phenomenon in segregating generations of wheat cross was reported by several investigators (Voigt and Tischler, 1994 and Al- Balkry *et al.*, 2008) expressed in much better performance than both parents and was the cause of developing high yielding varieties under some abiotic stresses (Al-Bakry and Al-Naggar, 2011, Al-Naggar and Shehab-Eldeen, 2012 and Al-Naggar *et al.* 2004, 2012, 2013).

The best ten DH lines (highest yielding under 9000 ppm NaCl and under 6000 ppm NaCl in this study ( L109, L11, L94, L103, L79, L81, L93, L28, L26, L24) are recommended for field evaluation in Egyptian salt affected areas,

hoping that one or more of them could show its superiority in grain yield and salinity tolerance, under the natural salinity conditions, over the best Egyptian cultivars recommended for these areas, such as Sakha-93.

## Conclusion

The results of this study concluded that tillers/ plant was the most sensitive trait, while grain yield was the most tolerant one to both salinity stresses (9000 and 6000 ppm NaCl). The 10 highest salinity tolerance index (STI) estimates under the three salinity stress treatments (3000, 6000 and 9000 ppm NaCl) were shown by L8, L9, L33, L84, L93, L10, L11, L2 and L109, in descending order under all studied salinity stress treatments. The highest yielding and most tolerant DH line (HY-T) under 9000 ppm NaCl was L109 followed by L11, L94, L103, L79 and L 81. The six best DH lines (highest yielding and most tolerant) under 9000 ppm NaCl in this study ( L109, L11, L94, L103, L79 and L81) that outyielded the best check cultivar (Sakha-93) by 186.3,168.1, 160.5, 157.0, 149.8 and 128.5%, respectively, were recommended for field evaluation in Egyptian salt affected areas, hoping that one or more of them could show its superiority in grain yield and salinity tolerance, under the natural salinity conditions, over the best Egyptian cultivars recommended for these areas, such as Sakha-93.

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