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Field Screening of Wheat (*Triticum aestivum* L.) Genotypes for Salinity Tolerance at Three Locations in Egypt

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMMA-N designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SRSS and MMMA managed the literature searches and analyses of the study. Author OMAE-A performed the experimental processes. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Although screening large numbers of wheat genotypes for salinity tolerance under controlled elevated salinity levels in the greenhouse is useful, the final screening in the field at different locations, where soils are naturally affected with salt and other uncontrolled factors in soil and climate interact with salinity, is a must before deciding the most suitable genotype for each location. In the present study, 117 bread wheat doubled haploid (DH) lines derived from the cross Sakha 8 X Line 25, along with their parents and the two check cultivars Sakha 93 and Sids 1 were screened for salinity tolerance under field conditions at three locations and two seasons, *i.e.* Serw (2011/12), Sakha (2011/12), Sakha (2013/14) and Gemmeiza (2013/14), where EC_e was 9.4, 5.7, 5.5 and 2.4 dSm⁻¹, respectively and irrigation water EC_w was 0.46 - 0.60 dSm⁻¹. The genotypes were classified into salt tolerant, moderately tolerant, sensitive and very sensitive based on grain yield/ plant. The rank of tolerant genotypes differed from one location to another and from season to season. The

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ten most tolerant DH lines across all environments were No.19, 44, 65, 33, 24, 2, 21, 98 61 and 99. The best DH line out-yielded the best check by 40.6% at Serw 11/12 (L40), 107.8% at Sakha 2011/12 (L16), 10.2% at Sakha 2013/14 (L2), 28.5% at Gemmeiza 2013/14 (L71) and 48.7% across the four environments (L19).

Keywords: Hexaploid wheat; salinity tolerance; field evaluation; doubled haploids; salt affected soils.

1. INTRODUCTION

In Egypt, more than 840,000 ha, *i.e.* about 25% of total cultivated lands are salt affected; these lands are located in North-, East- and West-Delta and some other areas in Wadi Al-Natron, Al-Tal Al-Kebeir, Al-Wahat and Al-Fayoum regions [1]. The reasons of such salinity in Egypt are: the low annual precipitation (less than 25 mm), the high temperature, especially in summer months (between 35 and 45°C), the surface evaporation that reaches 1500-2400 mm annually, non-proper irrigation systems in irrigated lands, the rise of ground water level to less than 1 m depth and its salinity to > 4.5 dSm⁻¹ [2].

Wheat (Triticum aestivum L.) is one of the oldest and most important cereal crops in Egypt. Although wheat 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\$ [3]. 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 suffers from salinity; therefore increasing salt tolerance for wheat genotypes is one of the cheap methods to enhance growing wheat in these areas.

Evaluation of wheat salt tolerance in field conditions is difficult due to high soil heterogeneity, making it complex to identify variations in genotypes. Pearce and Moore [4] and Bartlett [5] suggested several statistical techniques to resolve these problems, e.g., use of small blocks or adjusting values according to that of neighboring plots. These techniques had reduced variation due to error and enhanced detection of variations due to genotypes. Approaches utilized lately consist of use of long rows along salinity gradients, while differences within-row are detected with electromagnetic salinity sensors [6]. Crop evaluation under field conditions is the critical test of genotypes for

salinity tolerance. Field evaluation should be done at replicated sites. Field conditions differ greatly from place to place with respect to physical and chemical properties of soil. High soil pH inhibits uptake of K^+ although it may not influence Na⁺ uptake [7]. Boron influences salt distribution in leaves and also salinity tolerance of plant [8].

In Equpt. Salib et al. [9] evaluated in field the bread wheat cultivars Sakha 8, Sahel 1 and Sids 1 for salinity tolerance. They reported that Sakha 8 cultivar was the most tolerant for salinity stress. Ashraf and Shahbaz [10] screened twenty-five cultivars of early CIMMYT hexaploid wheat for salt tolerance in a glasshouse experiment and also in salt affected soil in the field. Every cultivar used its own specific mechanism to tolerate salt stress. However, a large amount of variation in salt tolerance observed in 25 early CIMMYT wheat cultivars can be of considerable practical value for improving salt tolerance in the existing commercial hexaploid wheats. Barrett-Lennard [11] reported that water logging aggravates the effects of salinity on wheat. It may be major cause of fact that wheat breeding for salt tolerance had less achievements in farmers' fields [12]. Plants having better salt tolerance should perform well in optimal soil conditions. El-Hendawy et al. [13] reported that tillers were more affected as compared to leaf number by salinity. Dry weight was reduced significantly at all growth stages by salinity. Spikelet on main tiller was reduced more by salinity than spike length, grain number and 1000 seed weight. He concluded that increase in spikelet spike⁻¹ and tiller plant⁻¹ could improve salinity tolerance in wheat cultivars. However, Francois et al. [14] reported that relative grain yields of wheat cultivars were unaffected by soil salinity up to 8.6 dSm⁻¹ EC. Every unit increase in salinity above the thresholds reduced the yield by 3%. Salinity reduced vegetative growth more than grain yield. Salam et al. [15] reported that number of tillers and grains, 100 seed weight and grain yield were adversely affected by salinity. Kingsbury and Epstein [16] evaluated 5000 wheat genotypes in 50% seawater and identified 29 accessions that produced seed. Jafari-Shabestari et al. [17] evaluated 400 wheat genotypes in field at

California and recognized numerous genotypes that always gave high yield under low and high salinity levels. Ahmad et al. [18] studied six wheat varieties in salt affected soils and narrated that salt tolerant varieties produced greater yield than salt susceptible due to higher dry weight of shoot and spike and better grain development. In India, Kamboj [19] studied twelve wheat genotypes under salinity conditions. Grain yield per plot and harvest index recorded the highest phenotypic and genotypic variations.

One hundred seventeen bread wheat doubled haploid (DH) lines derived from the cross Sakha 8 X Line 25 via anther culture technique [20] 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. The objectives of the present investigation were: (i) to identify the salt tolerant and high yielding DH lines under salt-affected field conditions for use in future breeding programs in Egypt and (ii) to calculate the superiority of the best DH lines over the best check cultivar(s).

2. MATERIALS AND METHODS

2.1 Plant Materials

Seeds of 121 bread wheat genotypes, including 117 doubled haploid lines, their two parents (Sakha-8 and Line-25) of the cross from which they were produced via anther culture technique and the 2 check cultivars Sids-1 and Sakha-93 obtained from Wheat Research were Department, Field Crop Research Institute (FCRI), Agricultural Research Center (ARC), Egypt. The reason of using this large number of lines in this study is to exploit the maximum variation resulted from the crossing between a salt tolerant cultivar (Sakha-8) and a high yielding promising (Line-25) in an attempt to find new line(s) that assemble more tolerance to salinity and higher yielding ability as compared to their parents and the check cultivars, i.e., new sources of salinity tolerance.

2.2 Sowing Method

The present investigation was carried out in the fields of following experimental research station of ARC:1. Sakha (Kafr-El-Sheikh governorate) at season 2011/2012, 2. Serw (Domiat governorate) at season 2011/2012, 3. Sakha (Kafr-El-Sheikh governorate) at season

2013/2014 and 4. Gemmieza (Gharbia governorate) at season 2013/2014. The stations are located at Sakha (31° 5 20` N, 30° 57 3` E and altitude = 2 m asl); Serw (31° 7 42° N, 30° 38 40° E and altitude = 6 m asl) and Gemmeiza (30° 58 20` N, 31° 23 20` E and altitude = 20 m asl). Sowing date was 29/11/2011 at Sakha, 22/11/2011 at Serw, 27/11/2013 at Sakha and 25/11/2013 at Gemmeiza station. A simple lattice design (11 X 11) with two replications was used. The seeds were sown in individual hills in rows. Each row was of 2.5 meter length and row to row distance was 30 cm and hill to hill distance was 10 cm. The irrigation and fertilization were done as recommended by ARC, for commercial production at the three locations. Flooding irrigation was done through the season; the first irrigation was given after 21 days from planting and the succeeding ones were given at 20 to 25 days intervals according to the weather conditions. The fertilization was applied using 15 kg P₂O₅ (100 kg Mono Super Phosphate 15.5%) + 70 kg Nitrogen (210 kg Ammonium Nitrate 33.5%) per feddan (one feddan=4200 m²) split in three parts, first 20% with seeds, second 40% with first irrigation and third time 40% with second irrigation. Soil and water analyses of the three locations were done in the Analysis and Studies Unit (ASU), Soil, Water and Environment Res. Inst. (SWERI) of ARC, Equpt and the data are presented in Table (1). The meteorological data at each location were recorded by Meteorological Station at each location (Table 2).

2.3 Data Recorded

Data were recorded on the following traits: Days to heading (DTH): It was estimated as the number of days from sowing date to the date at which 50% of main spike awns/plot had completely emerged from the flag leaf. Days to maturity (DTM): It was recorded as the number of days from sowing to the date at which 50% of main peduncles/plot have turned to yellow color (physiological maturity). Grain filling period (GFP): Number of days from 50% anthesis to 50% physiological maturity (on a per plot basis). Grain filling rate (GFR): It was calculated as the accumulated dry weight of grains per day as follows: GFR = GYPP / GFP. 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, as an average of five plants. Number of spikes/plant (NSPP): It was measured as the total number of fertile spikes per plant as an average of five plants. Number of grains/spike (NGPP): It was measured as the total number of grains per main spike, as an average of five spikes. Thousand grain weight (TGW): It was measured as the weight of 1000 grains using an electronic balance. Grain yield/plant (GYPP): It was measured as the dry matter (biomass) allocated to the grains per plant as an average of five plants. 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 five plants. Biological yield/plant (BYPP): It was measured as the dry matter (biomass) allocated to the whole plant, except root, as an average of five plants. It was estimated as follows: BYPP = GYPP + SYPP. Harvest index (HI): It was estimated as follows: HI = 100 (GYPP / BYPP).

2.4 Statistical Analysis

All data were subjected to analysis of variance (ANOVA) of lattice design (11 X 11) using GENSTAT 10^{th} EDITION FOR WINDOWS 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 [21]. Combined analyses of variance across locations and/or seasons were performed after testing the homogeneity of error.

3. RESULTS

Soil analysis of the three field soils revealed marked differences in EC_{e} (electrical conductivity) and soil texture (Table 1). Salt affected soils at Serw location showed the highest EC_e (9.4 dSm⁻¹) in the present investigation which is equal to 7520 ppm NaCl, while soils of Gemmeiza location revealed the lowest EC_e (2.4 dSm⁻¹) or 1536 ppm NaCl. The soils of Sakha location were in between Serw and Gemmeiza locations for ECe (5.7 and 5.5 dSm⁻¹) which is equal to 4560 and 4400 ppm NaCl in 2011/12 and 2013/14, respectively. The irrigation water at all locations was coming from the River Nile, where EC_w was between 0.46 and 0.60 dSm⁻¹ or 294 and 384 ppm NaCl. Four environments were therefore used namely. Serw (2011/12), Sakha (2011/12), Sakha (2013/14) and Gemmeiza (2013/14).

Table 1. Soil and water analyses at Serw, Sakha and Gemmeiza locations

Analysis		Se	erw		Sa	Gemmeiza			
		(2011	/2012)	(201 [,]	1/2012)	(2013	6/2014)	(2013/2	2014)
		Soil	Water	Soil	Water	Soil	Water	Soil	Water
Clay %		6.3		55.6		51.9		54.6	
Silt %		40.0		30.1		32.3		35.5	
Fine sand		30.4		8.8		10.8		6.4	
Coarse sand		23.3		5.5		5.0		3.5	
Soil type		Sandy L	₋oam	Clay		Clay		Clay	
рН		8.4	6.8	7.8	7.1	8.1	7.1	7.9	7.4
EC (dSm ⁻¹)		9.4	0.5	5.7	0.5	5.5	0.5	2.4	0.6
Salt Conc. (opm)	7520	294	4568	333	4400	333	1536	384
Soluble	Ca ⁺²	11.2	0.0	3.0	0.0	3.9	0.0	3.0	0.0
Cations	Mg ⁺²	12.6	0.0	3.4	1.0	3.2	0.9	2.4	0.0
mEqu/l	Na⁺	48.2	2.6	20.0	4.0	19.0	4.5	11.6	3.7
	K⁺	1.1	0.0	0.6	0.0	0.7	0.0	1.5	0.0
	Ca	6.0	0.0	3.0	0.4	3.0	0.5	1.9	0.4
Soluble	Cl	24.0	1.4	15.0	2.0	14.0	2.2	10.0	2.3
Anions	SO4	15.0	0.0	9.0	1.0	8.5	1.8	7.0	1.2
mEqu/l	CO_3^-	2.0	0.0	0.0	0.0	0.0	0.1	1.0	0.1
	NO_3^-	9.6	0.6	3.0	0.1	2.4	0.3	1.5	0.2
Macro eleme	ents ppm								
	N	20.0	0.0	50.0	0.0	42.0	0.0	55.0	0.0
	Р	3.0	0.0	15.0	0.0	16.0	0.0	14.0	0.0
	K	728.0	0.0	820.0	0.0	840.0	0.0	960.0	0.0
Micro eleme	nts ppm								
	Fe	23.0	0.0	40.0	0.0	43.0	0.0	48.0	0.0
	Cu	6.4	0.0	8.2	0.0	9.0	0.0	9.5	0.0
	Zn	0.9	0.0	1.9	0.0	2.1	0.0	2.0	0.0
-	Mn	13.2	0.0	18.8	0.0	19.8	0.0	19.9	0.0

Source: Analysis and Studies Unit, Soil, water and Environment Res. Inst. (SWER), ARC, Egypt

Month	Temp.	Temp.	Temp. mean	RH max.	RH	RH	Precipitation
	max.	min.	(0)	0/	min.	mean	()
0-1-1 (((C)	<u>(</u> (C)	(C)	%	%	%	(mm)
Sakna (4	2011/2012	<u>)</u>	10 5	00.4	50 7	07.5	•
NOV.	22.8	10.3	16.5	86.4	53.7	67.5	0
Dec.	21.3	6.8	13.6	86.5	61.6	72.8	14.6
Jan.	19	13.8	16.3	84.4	63.2	//.4	0
Feb.	11.4	9.6	10.5	76.8	61.3	69	32.7
Mar.	14	12.1	13.6	77.1	58.8	68	42.8
Apr.	19	17.1	18	73	52.6	63	0
Total							90
Sakha (2	2013/2014	.)					
Nov.	22.8	10.3	16.4	84.7	54.7	68.5	0
Dec.	20.7	6.8	12.5	86	61.3	71.6	21.4
Jan.	10.2	8.8	8.9	77.3	60	68.6	32.5
Feb.	11.4	8.8	9.7	80.2	67.4	74.1	50.4
Mar.	12.5	10.5	11.2	77.8	57.5	66.5	44.9
Apr.	19.4	16.8	17.9	72.3	51.5	63	0
Total							149.2
Serw (20	011/2012)						
Nov.	21.2	9.6	15.3	78.7	50.9	63.7	0
Dec.	20.2	7.2	12.8	81	54.7	65.9	16.9
Jan.	14.1	7.2	10.6	73.7	57.3	64.8	36.6
Feb.	9.6	8	8.3	73.7	57.9	65.7	58.7
Mar.	10.6	8.3	9	74.3	62	68.7	81.7
Apr.	11.6	9.8	10.4	72.3	53.5	61.8	41.8
Total							235.8
Gemmei	za (2013/	2014)					
Nov.	22.7	10.7	16.6	81.3	52.9	66	0
Dec.	22.2	9.2	14.5	82	55.5	66.4	17.3
Jan.	20.3	7.5	13.5	82.8	59.1	69.8	37.3
Feb.	11.7	8.8	11.6	74	58.3	66	59.9
Mar.	11.1	9.5	9.7	76.5	60.9	68.5	83.4
Apr.	11.8	9.5	10.2	76.8	64.2	71.1	42.6
Total							240.5

Table 2. Meteorological data during seasons of wheat growing at Serw (2011/2012), Sakha (2011/2012 and 2013/2014) and Gemmeiza (2013/2014)

Source: Meteorological Stations of Agric. Res. Centre at Serw, Sakha and Gemmeiza. R.H. = Relative humidity, Temp. = Temperature

Out of 121 wheat genotypes sown (117 doubled haploids, the two parents Sakha 8 and Line-25 and the two check cultivars; Sids-1 and Sakha-93), all genotypes reached maturity at all locations, except at Serw location, where 13 genotypes did not reach maturity. In 2011/12 season at Serw and Sakha locations, nine traits were studied (DTH, DTM, GFP, GFR, PH, NSPP, NGPS, TGW and GYPP) while in 2013/14 season at Sakha and Gemmeiza locations, three more traits were studied; namely SYPP, BYPP and HI.

3.1 Analysis of Variance

Combined analysis of variance across the four environments (Table 3) revealed that genotypes differed significantly ($P \le 0.01$) for all studied traits at all environments. Variance due to genotypes was the main contributor to the total variance in the present investigation; this was measured by the highest percentage of genotypes sum of squares to the total sum of squares (Table 3). Mean squares due to genotypes x environments interaction were significant ($P \le 0.05$ or 0.01).

To compare the effect of locations, that differ in salinity of their soils, with the genotypes effect, and their interaction, two combined analyses of variance (ANOVA) were performed; one across Serw and Sakha locations in 2011/12 season (Table 4) and another one across Gemmeiza and

Sakha locations in 2013/14 season (Table 5). The first contributor to the total variance in the combined ANOVA across Serw and Sakha locations in 2011/12 season was genotype for seven out of nine studied traits, but was location for one trait (number of grains/spike) and genotype x location interaction for one trait (number of spikes/plant) (Table 4). Mean squares due to genotypes x location interaction were significant for all traits, except for plant height trait in 2011/12 season, indicating that the rank of genotypes differ from Serw to Sakha location for 8 out of nine traits. For the combined ANOVA across Sakha and Gemmeiza locations in 2013/14 seasons (Table 5), mean squares due to locations were significant (P≤ 0.05 or 0.01) for all the 12 studied traits, except for PH and GYPP traits, indicating that variation of these locations affected on all studied traits except plant height and grain yield/plant. Mean squares due to genotypes and genotypes X locations interaction were significant ($P \le 0.05$ or 0.01) for all studied traits. indicating that genotypes differed significantly and their ranks differed from one location to another.

3.2 Genotypic Differences

Mean grain yield per plant (GYPP) of the studied wheat genotypes in the field showed a wide range, *i.e.* great differences between the minimum and maximum values under each of the four environments (Table 6). The DH line No.19 showed the highest GYPP (48.729) across all environments followed by the DH lines No. 44, 65, 33, 24, 2, 21, 98, 61, 99, 60 and 54 with GYPP of 46.98, 46.55, 45.42, 43.06, 42.36, 42.33, 42.11, 41.45, 41.19, 41.16 and 40.97 g, respectively which were significantly superior over the best check cultivar cross all environments Sakha 8 (39.88 g). On the contrary, the lowest DH line for GYPP across environments was No.105 (23.07 g) and the lowest check cultivar was Sakha 93 (28.59 g).

Means of four selected characters of all studied genotypes under each environment showed also great differences. The earliest DH line in maturity was L91 at Serw, L83 at Gemmeiza, L91 at Sakha (2011/12) and L97 at Sakha 2013/14, while the latest one was L16 at Serw, L9 at Gemmeiza, L116 at Sakha 2011/12 and Line 25 at Sakha 2013/14. The tallest DH line was L62 at Serw, L103 at Gemmeiza, L62 at Sakha 2011/12 and L31 at Sakha 2013/14, while the shortest line was L93, L31, L59 and L117 at Serw 11/12, Gemmeiza 13/14, Sakha 11/12 and Sakha 13/14, respectively. The highest number of spikes/plant was obtained by L61, L100, L65 and L73, while the lowest number was obtained by L112, L33, L10 and L33 at Serw, Gemmeiza, Sakha 2011/12 and Sakha 2013/14, respectively. The highest number of grains/spike was exhibited by L112, L66, L54 and L10, while the lowest NGPS was shown by L28, L31, L50 and L110 at Serw, Gemmeiza, Sakha 2011/12 and Sakha 2013/14, respectively. The heaviest grain was shown by L40, L71, L16 and L2 and the highest one by L43, L87, L82 and L31 under Serw, Gemmeiza, Sakha 2011/12 and Sakha 2013/14, respectively.

SOV	df		Su	m of squares	%	
		DTH	DTM	GFP	GFR	PH
Environments (E)	3	2.56	3.25	2.89	5.07	9.58
Error (a)	6	0.39	0.25	0.87	0.98	0.52
Genotypes (G)	120	53.2**	51.3**	49.2**	64.0**	62.2**
GXE	360	20.2**	30.3**	37.0**	26.5**	22.4**
Error (b)	720	23.59	14.97	9.99	3.50	5.31
Total SS		598764	213589	76982	8191	169785
		NSPP	NGPS	TGW	GYPP	
Environments (E)	3	11.25	15.58	12.36	8.69	
Error (a)	6	1.48	1.98	2.05	5.25	
Genotypes (G)	120	52.2**	48.2**	60.2**	54.1**	
GXE	360	32.9**	29.9**	22.4*	31.1**	
Error (b)	720	2.2	4.2	3.0	0.9	
Total SS		64236	512037	457820	26543	

Table 3. Combined analysis of variance of studied traits of 121 genotypes across four environments (Serw 11/12, Sakha 11/12 Sakha 13/14 and Gemmeiza 13/14)

SOV	df	Sum of squares %						
		DTH	DTM	GFP	GFR	PH		
Locations (L)	1	9.2*	7.4	4.41	3.78	3.11		
Error (a)	2	2.3	5.1	4.29	5.91	3.79		
Genotypes (G)	120	51.0**	47.0**	59.3**	52.6**	81.0**		
GXL	120	32.1**	32.0**	19.1**	34.6**	5.65		
Error (b)	240	6.4	8.5	12.95	3.11	6.48		
Total SS		524879.5	495479.3	150782.0	5365	181344.5		
		NSPP	NGPS	TGW	GYPP			
Locations (L)	1	9.5	57.66 **	0.056	0.34			
Error (a)	2	7.3	0.31	1.23	0.4			
Genotypes (G)	120	25.2**	19.7**	46.6**	66.4**			
GXL	120	51.3**	19.4**	41.3**	29.8**			
Error (b)	240	6.7	2.92	10.85	3.1			
Total SS		458723.9	359357.4	161836.4	79199			

 Table 4. Combined analysis of variance of studied traits of genotypes across two locations

 Serw and Sakha in 2011/2012 season

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

 Table 5. Combined analysis of variance of studied traits of genotypes across two locations

 Sakha and Gemmeiza at (2013/2014) season

SOV	df	Sum of squares %						
		DTH	DTM	GFP	GFR	PH	NSPP	
Location (L)	1	39.9**	20.4**	32.99	22.8**	1.99	19.5**	
Error (a)	2	0.096	0.66	2.19	1.93	2.19	2.95	
Genotypes (G)	120	47.9**	45.2**	46.5**	56.9**	66.5**	25.5**	
GXL	120	9.1*	26.0**	10.7*	15.6**	16.7*	18.8**	
Error (b)	240	2.99	7.79	12.58	3.78	12.58	33.3**	
Total SS		10856	3093	5847	2499	42234	82624	
		NGPS	TGW	GYPP	SYPP	BYPP	HI	
Location (L)	1	75.2**	9.18*	0.26	65.6**	53.5**	59.2**	
Error (a)	2	0.01	4.62	0.0038	1.38	1.13	0.19	
Genotypes (G)	120	8.0*	28.9**	31.5**	9.8*	13.9*	12.8*	
GXL	120	6.5*	21.5**	27.7**	7.0*	10.0*	10.4*	
Error (b)	240	10.3*	35.9**	40.5**	16.3**	21.4**	17.4**	
Total SS		175682	140931	66929	7366990	868982	52869	

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

3.3 Grouping Genotypes for Salt Tolerance in the Field

Based on GYPP, the studied DH lines and checks were classified into four groups, *i.e.* tolerant (T), moderately (MT), sensitive (S) and very sensitive (VS) under each environment. The difference between the maximum and minimum value at each location was divided into four equal groups (Table 7), namely, salt tolerant (average yield 41.50, 42.80, 61.30, 68.60 and 42.30 g/plant and above), moderately salt tolerant (average yield 33.59, 33.64, 55.68, 56.30 and 38.75 g/plant), salt susceptible (average yield

21.85, 23.45, 47.55, 42.93 and 33.16 g/plant) and salt very susceptible (average yield below 14.9, 17.3, 42.4, 35.9 and 29.5 g/plant) for Serw 11/12, Sakha 11/12, Sakha 13/14, Gemmeiza 13/14 and combined across all environments, respectively. Number of tolerant genotypes was 8, 9, 8, 5 and 7 at Serw 2011/12, Sakha 2011/12, Sakha 2013/14, Gemmeiza 2013/14 and combined across environments, respectively. The DH No. 19 was tolerant or moderately tolerant in the four environments, the DH lines 21 and 24 were tolerant or moderately tolerant at three environments (Serw, Sakha 2011/12 and Gemmeiza), lines 33 and 65 at Serw, Sakha

2011/12 and Sakha 2013/14, lines 17 and 44 at Sakha 2011/12 Sakha 2013/14 and Gemmeiza 2013/14 and line No. 61 at the three Serw, Sakha 2013/14 environments and Gemmeiza 2013/14. In the two environments Serw 2011/12 and Sakha 2011/12, the DH lines 59, 69 and 98 were tolerant or moderately tolerant. Sakha 8 cultivar was tolerant or moderately tolerant at two environments (Sakha and Gemmeiza in 2013/2014 season), and Sids 1 cultivar was tolerant at Serw in 2011/2012 season. The studied genotypes (DH lines and checks) were grouped according to their grain vield under the control environment (Gemmeiza 2013/14) (responsive vs. non responsive) as well as under the elevated level of salinity at Sakha 2013/14, and Sakha and Serw 2011/12) (efficient vs. non-efficient) genotypes (Figs. 1, 2 and 3, respectively). Grain yield/plant under Serw 2011/12 (7520 ppm) vs. Gemmeiza 2013/14 (1536 ppm) (Fig.1) grouped the genotypes into 25 efficient responsive (E-R) genotypes, 25 efficient non-responsive (E-NR) genotypes, 31 non-efficient responsive (NE-R) genotypes and non-efficient non-responsive (NE-NR) genotypes and non-efficient non-responsive (NE-NR) genotypes. Under Sakha 2011/12 vs. Gemmeiza 2013/14, grain yield/plant grouped the genotypes into 27 E-R, 23 E-NR, 34 NE-R and 37 NE-NR genotypes (Fig. 2). Grain yield/ plant under Sakha 2013/14 vs. Gemmeiza 2013/14 grouped the genotypes into 28 E-R, 25 E-NR, 33 NE-R and 35 NE-NR genotypes.

3.4 Superiority of DH Lines over the Best Check

The number of DH lines exhibiting significant (P \leq 0.01 or 0.05) superiority in GYPP over the best check was 9 at Serw (2011/12). 29 at Sakha 2011/12, 3 at Sakha 2013/14, 9 at Gemmeiza 2013/14 and 3 for combined data across environments (Table 8). These DH lines were L40, L102, L89, L63, L27, L98, L24, L59 and L61 at Serw, L16, L117, L44, L60, L54, L19, L87, L21, L38, L85, L76, L33, L84, L65, L2, L69, L24, L47, L59, L114, L94, L42, L91, L12, L15, L11, L57, L98 and L23 at Sakha 2011/12, L2, L6 and L107 at Sakha 2013/14. L71. L101. L108. L38. L96, L36, L80, L49 and L85 at Gemmeiza 2013/14 and L19, L44 and L65 for combined data across environments. The best DH line outyielded the best check by 40.6% at Serw (L40), 107.8% at Sakha 2011/12 (L16), 10.2% at Sakha 2013/14 (L2), 28.5% at Gemmeiza 2013/14 (L71) and 18.1% for combined data across all environments (L19).

4. DISCUSSION

Our results revealed that the 121 wheat genotypes (117 DH lines, their two parents and two check cultivars) evaluated in the field at different salt stressed locations in Egypt varied greatly in all studied agronomic and yield traits. Mean squares due to genotypes x environments interaction were significant ($P \le 0.05$ or 0.01), indicating that selection is possible under each specific environment, and therefore the proper genotype could be indentified for each environment of specific salt affected soil. This conclusion is in agreement with that reported by previous investigators, e.g. [22,23,24]. Our results revealed that wheat genotypes responded differently to salinity stress at the three locations in terms of yield and yield components. Similar findings were reported by [22,23,25]. Significance of genotype X location interaction variances in both combined analyses (Tables 3 and 4) indicated that selection in each location (specific soil EC_e) could be efficient in isolating the best genotype(s) for each specific location. Thus, wheat genotypes behaved differently under different salt affected soil conditions and the superior genotype under one location might be different under another location, and a specific genotype should be identified as the best one in each location of a specific salt affected soil. Similar findings were also reported by [22,23,25,26].

Salt tolerance of genotypes under field conditions needs to be evaluated particularly as a function of yield that is considered as a foremost target of the plant breeder [27]. Kingsbury and Epstein [16] evaluated 5000 accessions of bread wheat in 50% seawater and identified 29 accessions that produced seed. Jafari-Shabestari et al. [17] evaluated 400 Iranian wheat genotypes in irrigated field conditions in California and identified numerous accessions that were consistently high for grain yield in both low and high salinity treatments. Ahmad et al. [18] studied six wheat varieties in salt affected soils and reported that salt tolerant varieties produced greater yield than salt susceptible due to higher dry weight of shoot and spike and better grain development. El-Hendawy et al. [28] evaluated wheat genotypes and reported that grain weight plant⁻¹, number of grains plant⁻¹ and number of fertile spikes plant⁻¹ are good screening criteria under field conditions. Our results revealed that wheat genotypes responded differently to salinity stress at the three locations in terms of yield and yield components. Similar findings were reported by [22,23].

No. DH	Serw	Sakha	Sakha	Gemm	No.	Serw	Sakha	Sakha	Gem
line	11/12	11/12	13/14	eiza	DH	11/12	11/12	13/14	meiza
				13/14	line				13/14
1	27.3	15.9	57.7	41.7	45	18.6	25.5	37.9	22.3
2	19.5	36.1	69.8	44.0	46	16.8	24.2	42.0	31.0
3	36.7	22.6	45.3	41.0	47	0.0	33.1	41.6	58.5
4	14.4	11.4	46.8	44.8	48	0.0	9.0	47.0	49.4
5	5.7	15.6	56.2	58.2	49	23.1	30.2	42.5	62.9
6	14.3	14.2	66.7	51.2	50	4.1	20.6	50.6	52.5
7	14.6	23.6	41.1	55.7	51	14.0	24.5	36.9	40.1
8	9.1	24.0	53.8	48.9	52	5.8	28.1	52.8	47.7
9	11.8	27.6	55.2	47.6	53	7.0	25.5	43.1	55.0
10	5.4	19.4	51.6	37.3	54	22.4	46.6	40.3	54.6
11	5.0	31.1	48.6	37.0	55	0.0	24.6	55.2	46.3
12	6.2	31.8	51.2	34.9	56	0.0	29.5	40.1	54.1
13	14.9	14.9	48.1	55.5	57	23.8	31.0	51.5	41.1
14	0.0	27.5	47.9	22.2	58	31.8	25.8	43.5	44.0
15	8.5	31.6	43.0	40.8	59	41.5	33.1	46.7	41.0
16	6.6	53.2	46.6	48.3	60	10.3	49.1	53.8	51.5
17	5.7	30.1	60.5	58.6	61	41.0	17.1	53.5	54.2
18	3.0	17.3	35.7	41.2	62	24.0	22.0	60.4	38.9
19	36.3	45.4	62.0	51.2	63	44.2	23.3	50.0	42.2
20	3.4	13.8	37.4	57.6	64	20.5	24.6	55.0	47.8
21	28.3	44.2	45.1	51.8	65	37.0	36.8	62.8	49.6
22	0.0	18.1	48.1	48.1	66	22.2	27.9	35.5	48.8
23	4.4	30.4	51.4	53.0	67	6.3	21.1	41.5	36.7
24	41.5	35.3	41.9	53.5	68	11.0	13.7	47.0	42.8
25	32.9	20.8	42.8	36.1	69	30.9	35.6	47.2	47.9
26	11.5	17.2	49.4	61.0	70	25.9	17.2	47.7	55.9
27	42.8	19.7	45.5	31.8	71	10.9	24.1	42.6	81.1
28	3.1	12.2	52.4	47.3	72	30.6	22.5	47.6	49.0
29	0.0	28.9	54.4	42.3	73	21.8	15.3	46.4	42.1
30	28.1	16.7	56.4	53.6	74	26.4	27.8	41.7	46.5
31	3.8	16.9	33.3	39.9	75	5.5	18.8	45.0	33.7
32	0.0	23.1	46.7	36.7	76	23.0	39.3	55.1	39.6
33	36.4	38.2	61.5	45.5	77	0.0	17.6	50.1	44.6
34	25.3	17.1	45.0	40.0	78	30.0	28.2	47.5	47.7
35	30.5	18.5	48.7	46.4	79	3.3	29.0	59.6	54.4
36	25.2	25.7	46.1	64.5	80	7.4	26.6	44.3	64.4
37	39.1	20.0	47.0	40.1	81	0.0	27.5	34.9	34.1
38	5.8	42.8	40.8	70.2	82	12.0	5.3	55.0	52.1
39	30.6	19.3	39.2	54.1	83	17.2	26.5	45.2	41.0
40	54.0	8.7	39.2	59.2	84	15.8	37.9	40.6	40.7
41	12.4	27.9	62.6	37.2	85	0.0	41.0	60.3	62.6
42	23.3	32.8	42.6	43.1	86	10.2	20.1	54.5	36.6
43	2.6	24.0	51.4	58.7	87	16.8	44.8	48.8	20.8
44	24.0	49.5	58.8	55.6	88	5.4	29.2	46.8	57.7

Table 6. Mean grain yield/ plant (g) of studied DH lines in the field at Serw, Sakha and
Gemmeiza locations in two seasons

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Table 6	. Cont.								
No. DH line	Serw 11/12	Sakha 11/12	Sakha 13/14	Gemm eiza 13/14	No. DH line	Serw 11/12	Sakha 11/12	Sakha 13/14	Gem meiza 13/14
89	44.6	25.5	56.1	37	107	27.4	17.3	65.9	46.4
90	7.6	14	39.8	55.1	108	2.8	23.8	53.4	70.3
91	16.6	32.4	56.2	36.2	109	6.5	25.3	40.9	37.0
92	14.5	15.6	46.6	47.1	110	0.0	21.6	38.3	43.6
93	15.9	18.6	43.5	37.3	111	25.2	19.2	57.4	40.2
94	18.9	32.9	51.3	44.8	112	20.8	23.5	54.3	36.0
95	6.5	27.0	55.0	32.7	113	31.2	15.9	48.7	59.7
96	11.5	15.4	46.4	68.6	114	8.7	33.0	50.7	56.7
97	9.7	23.7	44.8	42.9	115	21.0	17.8	46.0	47.8
98	42.8	30.6	43.7	51.3	116	11.5	18.0	41.1	58.7
99	23.8	28.1	56.2	56.7	117	13.7	50.6	49.4	39.3
100	8.5	20.4	37.6	44.0	Sk. 8	16.0	22.8	62.7	58.0
101	0.0	20.1	45.5	70.6	L. 25	8.0	23.7	43.2	49.5
102	45.8	10.4	35.2	37.7	Sd.1	38.4	15.3	47.0	48.8
103	29.3	16.1	41.2	33.2	Sk. 93	13.1	25.6	43.6	32.1
104	9.5	14.5	54.0	54.3	Mean	16.9	25.0	47.2	48.4
105	3.2	12.2	43.8	33.1	Max	54.0	53.2	81.1	69.8
106	22.7	22.1	47.7	47.7	Min	2.6	5.3	20.8	33.3

 Table 7. Salt tolerant categories of 117 DH Lines and 4 checks based on GYPP (g) under different environments

Salt tolerance category	No. of geno- types	DH lines/cultivars	Mean GYPP (g)	Range GYPP (g)
Serw (2011	I/2012)			
Т	8	40, 102, 89, 63, 27, 98, 24, 59.	44.65	41.5 - 54.0
МТ	17	61, 37, Sids 1 , 65, 3, 33, 19, 25, 58, 113, 69, 39, 72, 35, 78, 103, 21.	33.59	28.30 - < 41.0
S	32	30, 107, 1, 74, 70, 34, 36, 111, 44, 62, 57, 99, 42, 49, 76, 106, 54, 66, 73, 115, 112, 64, 2, 94, 45, 83, 46, 87, 91, Sakha 8 , 93, 84.	21.85	15.8 - < 27.0
VS	64	13, 7, 92, 4, 6, 51, 117, Sakha 93 , 41, 82, 9, 26, 96, 116, 68, 71, 60, 86, 97, 104, 8, 114, 15, 100, Line 25 , 90, 80, 53, 16, 95, 109, 67, 12, 38, 52, 5, 17, 75, 10, 88, 11, 23, 50, 31, 20, 79, 105, 28, 18, 108, 43, 14, 22, 29, 32, 47, 48, 55, 56, 77, 81, 85, 101, 110.	8.25	0.0 - < 14.9
Sakha (201	1/2012)			
Т	9	16, 117, 44, 60, 54, 19, 87, 21, 38.	37.36	42.8 - 53.2
MT	23	85, 76, 33, 84, 65, 2, 69, 24, 47, 59, 114, 94, 42, 91, 12, 15, 11, 57, 98, 23, 49, 17, 56.	33.64	29.5 - < 41.0
S	62	88, 79, 29, 78, 52, 99, 41, 66, 74, 9, 14, 81, 95, 80, 83, 58, 36, Sakha 93 , 45, 53, 89, 109, 55, 64, 51, 46, 71, 8, 43, 108, 97, Line 25 , 7, 112, 63, 32, Sakha 8 , 3, 72, 106, 62, 110, 67, 25, 50, 100, 86, 101, 37, 27, 10, 39, 111, 75, 93, 35, 22, 116, 115, 77, 18, 107.	23.45	17.3 - < 29.3
VS	27	26, 70, 34, 61, 31, 30, 103, 1, 113, 5, 92, 96, 73, Sids 1 , 13, 104, 6, 90, 20, 68, 28, 105, 4, 102, 48, 40, 82.	14.13	5.3 - < 17.3

Sakha (2	2013/2014)			
Т	8	2, 6, 107, 65, Shakha 8 , 41, 19, 33, 17.	64.25	61.3 - 69.8
МТ	29	62, 85, 79, 44, 1, 111, 30, 5, 99, 91, 89, 55, 9, 76, 82,	55.68	53.6 - < 60.3
		95, 64, 86, 29, 112, 104, 8, 60, 61, 108, 52, 28.		
S	57	10, 57, 23, 43, 94, 12, 114, 50, 77, 63, 117, 26, 87,	47.55	42.4 - < 51.6
		35, 113, 11, 22, 13, 14, 70, 106, 72, 78, 69, 48, Sids		
		1 , 37, 68, 4, 88, 59, 32, 92, 16, 73, 96, 36, 115, 27,		
		101, 3, 83, 21, 34, 75, 97, 80, 105, 98, Sakha 93, 93,		
		58, Line 25, 53, 15, 25, 71, 42, 49.		
vs	26	46, 24, 74, 47, 67, 103, 7, 116, 109, 38, 84, 54, 56,	39.06	33.3 - < 42.4
	(0040/00	<u>90, 40, 39, 110, 45, 100, 20, 51, 18, 66, 102, 81, 31.</u>		
Gemme	iza (2013/20)14)		
<u> </u>	5	71, 101, 108, 38, 96,	72.2	68.6 - 81.1
MT	39	36, 80, 49, 85, 26, 113, 40, 43, 116, 17, 47, 5,	56.3	53.1 - < 64.5
		Shakha 8, 88, 20, 99, 114, 70, 7, 44, 13, 90, 53, 54,		
		79, 104, 61, 56, 39, 30, 24, 23, 50, 82, 21, 60, 98, 19,		
		0.		
S	65	65, Line 25, 48, 72, 8, Sids 1, 66, 16, 22, 69, 64,	42.93	35.9 - < 51.0
		115, 106, 52, 78, 9, 28, 92, 74, 35, 107, 55, 33, 94, 4, 77, 2, 58, 100, 110, 42, 07, 68, 20, 62, 72, 1, 18, 57		
		<i>11</i> , 2, 30, 100, 110, 42, 97, 00, 29, 03, 73, 1, 10, 37, 3, 83, 50, 15, 84, 111, 51, 37, 34, 31, 76, 117, 62		
		102 10 93 41 11 109 89 32 67 86 91 25 112		
VS	12	12 81 75 103 105 95 Sakba 93 27 46 45 14	30 15	20.8 - < 35.9
٧J	12	87	50.15	20.0 - < 33.3
Combin	ed			
T	7	19 44 65 33 24 2 21	45.06	42.3 - 48.7
MT	39	98 61 99 60 54 85 89 59 69 36 40 63 38	38 75	36.0 - < 42.1
	00	Shakha 8 , 71, 49, 107, 76, 113, 17, 30, 16, 78, 117,	00.70	00.0 - 442.1
		108. 72. Sids 1 . 114. 94. 64. 57. 70. 6. 79. 37. 3. 62.		
		58, 35.		
S	50	39, 1, 80, 74, 9, 111, 96, 42, 91, 106, 41, 27, 23, 88,	33.16	29.5 - < 35.9
		26, 43, 101, 5, 8, 84, 7, 112, 52, 66, 13, 47, 25, 115,		
		104, 87, 53, 83, 116, 102, 50, 34, 55, 29, 73, Line 25,		
		82, 12, 15, 92, 56,11, 86, 95, 97, 103.		
S	25	4, 90, 51, 93, 28, 68, Sakha 93 , 22, 46, 10, 20, 77,	27.01	23.1 - < 29.5
		100, 109, 32, 67, 48, 45, 110, 75, 14, 18, 81, 31, 105.		
	T=	Tolerate, MT= Moderately tolerant, S= Sensitive, VS= Very se	ensitive	

Based on GYPP, the studied DH lines and checks were classified into four groups, *i.e.* tolerant (T), moderately (MT), sensitive (S) and very sensitive (VS) under each environment (Table 7). Moreover, grain yield/plant under Serw 2011/12 (7520 ppm) vs. Gemmeiza 2013/14 (1536 ppm) (Fig. 3) grouped the genotypes into 25 efficient responsive (E-R) genotypes, 25 efficient non-responsive (E-RR) genotypes, 31 non-efficient responsive (NE-R) genotypes and non-efficient non-responsive (NE-NR) genotypes. The first group (E-R) and the second group (E-NR) of genotypes are recommended for use under the salt stressed environment, e.g.

Serw and the third group NE-R would be recommend for the non stressed environment (Gemmeiza) only. It is worthy to note that the DH lines No. 19, 44, 65 and 99 are common E-R (efficient and responsive) genotypes across all environments (Figs, 3, 4 and 5). They are amongst the ten most tolerant DH lines across all environments (19, 44, 65, 33, 24, 2, 21, 98, 61 and 99) (Table 7). Further field evaluation of these DH lines should be carried out under salt stressed environments of soil $EC_e > 7$ at different locations in Egypt for at least two seasons, in order to get firm results and reduce the error resulting from the strong interaction between genotype and salinity status.



Fig. 1. Relationships between mean of grain yield/plant wheat genotypes under Gem. 13/14 (1536 ppm) and Serw 11/12 (7520 ppm), Broken lines represent means of GYPP (number from 1 to 108 refer to genotype name)

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Fig. 2. Relationships between mean of grain yield/plant wheat genotypes under Gem. 13/14 (1536 ppm) and Sakha 11/12 (4560 ppm), Broken lines represent means of GYPP (number from 1 to 121 refer to genotype name)

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Fig. 3. Relationships between mean of grain yield/plant wheat genotypes under Gem. 13/14 (1536 ppm) and Sakha 13/14 (4400 ppm). Broken lines represent means of GYPP (number from 1 to 121 refer to genotype name)

DH line	Mean	Super. %	DH line	Mean	Super. %	DH line	Mean	Super. %
no.	g/plant		no.	g/plant		no.	g/plant	
Serw 2011	/2012							
Best chec	k = Sids 1	(38.40 g/pla	ant)					
40	54.0	40.6**	63	44.2	15.1**	24	41.5	8.1*
102	45.8	19.3**	27	42.8	11.5**	59	41.5	8.1*
89	44.6	16.1**	98	42.8	11.5**	61	41.0	6.8*
Sakha 201	1/2012							
Best chec	k = Sakha	93 (25.60 g	g/plant)					
16	53.2	107.8**	76	39.3	35.5**	94	32.9	28.5*
117	50.6	97.7**	33	38.2	49.2**	42	32.8	28.1*
44	49.5	93.4**	84	37.9	48.0**	91	32.4	26.6*
60	49.1	91.8**	65	36.8	43.8**	12	31.8	24.2*
54	46.6	82.0**	2	36.1	41.0**	15	31.6	23.4*
19	45.4	77.3**	69	35.6	39.1**	11	31.1	21.5*
87	44.8	75.0**	24	35.3	37.9**	57	31.0	21.1*
21	44.2	72.7**	47	33.1	29.3**	98	30.6	19.5*
38	42.8	67.2**	59	33.1	29.3**	23	30.4	18.8*
85	41.0	60.2**	114	33.0	28.9**			
Sakha 201	3/2014							
Best chec	k = Sakha	8 (62.67 g/p	olant)					
2	69.8	10.2*	6	66.7	6.1*	107	65.9	5.0*
Gemmeiza	a 2013/201	4						
Best chec	k = Sakha	8 (58.04 g/	olant)					
71	81.1	28.5**	38	70.2	17.4**	80	64.4	9.8*
101	70.6	17.8**	96	68.6	15.4**	49	62.9	7.7*
108	70.3	17.4**	36	64.5	10.0*	85	62.6	7.2*
Combined								
Best chec	k = Sakha	8 (39.88 g/	olant)					
19	48.7	18.1**	44	47.0	15.1*	65	46.5	14.3*
* and	** indicate s	significant at 0	.05, 0.01 pro	obability lev	els, respective	ely. Super. %	6 = Superior	rity % =

Table 8. Superiority of the best DH lines over the best check under each environment and
combined across the four environments

[(Mean of line / Best check) -1]*100

The best DH line out-yielded the best check by 40.6% at Serw (L40), 107.8% at Sakha 2011/12 (L16), 10.2% at Sakha 2013/14 (L2), 28.5% at Gemmeiza 2013/14 (L71) and 18.1% for combined data across all environments (L19). Such high superiority of the new DH lines used in the present study might be attributed to the transgressive segregation in the progeny of the cross between the salt tolerant Egyptian cultivars Sakha 8 and the high-yielding promising line (Line 25) from which these DH lines originated via anther culture. The role of transgressive segregation in producing superior genotypes was reported in wheat by several investigators [29,30, 31,32,33,34,35].

Moreover, the role of doubled haploid technique in developing new varieties proved a great success in developing improved and perfect homozygous genotypes in many countries such as China, France, Hungary and Canada [36,37, 38].

5. CONCLUSION

Field evaluation at different salt affected locations in Egypt grouped the studied doubled haploid (DH) lines of wheat into four groups; tolerant, moderately tolerant, sensitive and very sensitive based on grain yield/plant (GYPP). The rank of genotypes was different under different locations. The four DH lines L 19, L 44, L 65 and L 99 were high yielding and tolerant across all environments. Some DH lines were superior over the best check cultivar at each location. Their superiority in GYPP reached 40.6% for L40 at Serw, 107.8% for L16 at Sakha 11/12, 10.2% for L2 at Sakha 13/14, 28.5% for L71 at Gemmeiza 13/14 and 47.8% for L19 across environments. This study recommended further field evaluation at different salt affected locations in Equpt for at least 2-3 seasons to get firm results on the best DH lines for each specific salt affected location.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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