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Assessment of Crossability between Tetraploid and Hexaploid Wheat Genotypes and Evaluating their Hybrids for Salinity Tolerance



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ABSTRACT

The present study aimed to investigate the crossability differences among three tetraploid durum and three hexaploid bread wheat genotypes and to study the chromosome number and meiotic behavior of their pentaploid F₁ hybrids and F₂ plants. The parental genotypes and their F₁ hybrids were also evaluated for salinity tolerance at seedling stage under 0 and 100 mM NaCl concentrations. The results showed high significant differences in the crossability (%) among the interspecific crosses as well as between direct and reciprocal crosses. The crossability (%) was high when the tetraploid species were used as maternal parents (direct crosses). The pentaploid F₁ hybrids had a chromosome complement of 35 chromosomes and showed abnormal meiotic behavior in different stages of meiosis. Cytogenetic analysis of F₂ plants revealed high variations in chromosome number within and among the tested F₂ populations, however some plants with 2n = 42 chromosomes were recorded. On the other hand, salinity stress affected durum wheat parents and their tetraploid hybrids higher than its effect on hexaploid wheat parents and their hexaploid hybrids for all studied traits. However, in general, pentaploid F₁ hybrids showed moderate reductions for all studied traits compared to their parents. Additionally, they were more tolerant to salinity as compared to their tetraploid parents, suggesting that salinity tolerance genes of the bread wheat parents were transmitted to their pentaploid F₁ hybrids. Thereby, the pentaploid hybrid strategy used in the present study could be an effective tool to transfer desirable genes and traits between tetraploid and hexaploid wheat species.

Keywords: Bread wheat, crossability, durum wheat, pentaploid hybrids, salt tolerance, seedling traits.

INTRODUCTION

Tetraploid durum wheat (2n= 28, genomes AABB) and hexaploid bread wheat (2n= 42, genomes AABBDD) are cultivated in various regions of the world (Shimelis and Spies 2011). Genetic differences between durum and bread wheat are due to the presence of D genome in hexaploid wheat and allelic differences at loci of the A and B genomes between durum and bread wheat (Kalous *et al.*, 2015). The genetic variability combined from tetraploid and hexaploid wheat in pentaploid hybrids has the potential to improve disease resistance (Martin *et al.*, 2013) and abiotic stresses tolerance such as salinity (Han *et al.*, 2014) and metal toxicity (Han *et al.*, 2016). Also it has the potential to enhance different agronomic traits in wheat (Kalous *et al.*, 2015; Deng *et al.*, 2018). And recently, a pentaploid crossing strategy via interspecific hybridization between tetraploid and hexaploid wheat is being increasingly considered as an efficient tool for transferring desired genes and traits in either direction (Deng *et al.*, 2018; Padmanaban *et al.*, 2018; Othmeni *et al.*, 2019).

Although the differences in ploidy levels between durum and bread wheat lead to variable degrees of sterility (Lanning *et al.*, 2008), successful establishment of interspecific hybrids has been long reported in wheat (Sharma and Gill 1983; Jiang *et al.*, 1993; Friebe *et al.*, 1996). However, the efficient production of pentaploid wheat hybrids remains a major challenge to wheat breeders (Bhagyalakshmi *et al.*, 2008); it faces several barriers such as low pollen compatibility, poor seed set and establishment and frequent sterility in F₁ hybrids

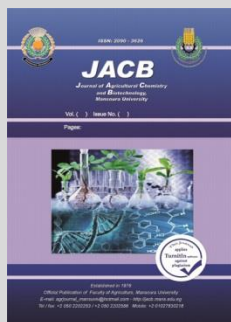
(Padmanaban *et al.*, 2017). In this regard, several studies have been achieved to overcome these barriers by careful selection of wheat cultivars (Hassan *et al.*, 2016) or even selection of paternal and maternal genotypes according to their ploidy level (Bhagyalakshmi *et al.*, 2008; Naskidashvili *et al.*, 2012) to be used for hybridization.

On the other hand, salinity is a serious problem in arid and semi-arid areas worldwide including Egypt affecting crops growth and productivity. Wheat is one of the main crops in Egypt and other countries which facing salinity problem and according to CIMMYT records, there are about 8–10% of the wheat planted areas in Egypt, Libya, Mexico, Iran, Pakistan, and India are affected by salinity (Mujeeb-Kazi and Diaz de Leon 2002). Therefore, genetic improvement for salt tolerance in wheat is required. However, classical breeding methods for salt tolerance in wheat have remained limited so far due to some factors such as: 1) mechanism of salt tolerance is complex and not fully understood, 2) differences in salinity tolerance in the different growth stages and 3) there are many physiological and morphological parameters that contribute to salt tolerance lead to the low efficiency of selection using multiple parameters (Ragab and Taha 2016). Alternatively, considering the genetic differences between and within durum and bread wheat cultivars (Munns *et al.*, 2000; Lindsay *et al.*, 2004; El-Hendawy *et al.*, 2019; Al-Ashkar *et al.*, 2020; Bacu *et al.*, 2020), it seems that screening durum and bread wheat genotypes and their pentaploid hybrids regard to their salinity tolerance could provide a great potential to improve salt tolerance in wheat breeding programs.

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The present study is a multipurpose study and aimed to: 1) assess the crossability differences between three tetraploid durum and three hexaploid bread wheat genotypes in order to identify the best cross combination and investigate the differences between direct and reciprocal crosses; 2) establish pentaploid hybrids to be used as intermediates for reciprocal introgression of useful traits between tetraploid and hexaploid wheat 3) develop monosomic lines for the D-genome of wheat to be used in the future in wheat breeding programs; 4) study the effect of salinity on three durum and three bread wheat genotypes and their F₁ hybrids at seedling stage.

MATERIALS AND METHODS

Plant materials

The initial plant material which used as parents in the present study included three tetraploid durum (*Triticum turgidum* L. var. *durum*) wheat genotypes viz. Sohag-3, BeniSuef-5 and Svevo and three hexaploid bread (*T. aestivum* L.) wheat genotypes viz. Sakha-8, Line-6 and Misr-2 (Table 1). The experiments were carried out at Genetics Department and the experimental farm of Faculty of Agriculture, Assiut University, Egypt.

Table 1. Names, genomes, pedigree and origin of durum and bread wheat genotypes.

No.	Name	Genome	Pedigree	Origin
P ₁	Sohag-3	AABB	MEXICALI/MAGHREBI 72//51792/DURUM#6	Egypt
P ₂	BeniSuef-5	AABB	DIPPER-2/ BUCHEN-3	Egypt
P ₃	Svevo	AABB	Cimmyt's Line/Zenit	Italy
P ₄	Sakha-8	AABBDD	CNO67//SN64/KLRE/3/8156	Egypt
P ₅	Line-6	AABBDD	Advanced long-spike inbred line	Egypt
P ₆	Misr-2	AABBDD	SKAUZ/BAV 92	Egypt

Field experiments

In the 2014/2015 winter season, the parental genotypes were sown in the field at two sowing dates with two weeks interval (25th November and 10th December) in order to synchronize the flowering for crossing purposes. The parental genotypes were crossed in all possible combinations to produce three tetraploid, three hexaploid and eighteen interspecific pentaploid F₁ hybrids; the pentaploid hybrids consist of nine pentaploid hybrids from direct crosses (using tetraploid genotypes as females) and nine pentaploid hybrids from reciprocal crosses (using hexaploid genotypes as females).

In the 2015/2016 winter season, seeds of the parents and their F₁ hybrids were sown in the field and their pollen mother cells were examined cytogenetically in order to confirm their chromosome number and to study their meiotic behavior. In the meantime, pentaploid F₁ progenies were also allowed to self-pollinate to produce F₂ populations.

In 2016/2017 winter season, the parental genotypes and six F₂'s populations derived from the pentaploid hybrids (Sohag-3 × Sakha-8, BeniSuef-5 × Sakha-8, Svevo × Sakha-8, Sohag-3 × Misr-2, BeniSuef-5 × Misr-2 and Svevo × Line-6) were field evaluated at optimum sowing date (24th November) in a randomized complete block design (RCBD) with three replications. The parents and their six F₂ populations were represented in each block by two and ten or twelve rows, respectively. Rows were three meters long, spaced 30 cm apart with seeds spaced 30 cm from each other. Measurements of plant height (cm) and spike length (cm) were recorded on individual plants basis. The percentages of germination and fertility in F₂ plants

were also recorded; the plants which failed to produce seeds were considered sterile.

In 2017/2018 winter season, the parents were sown in the field at two sowing dates with two weeks interval (25th November and 10th December) and were crossed to produce three tetraploid, three hexaploid and nine pentaploid F₁ hybrids which were produced from the direct crosses to be used for evaluating their salinity tolerance. Additionally, the progeny of F₂ population with 29 and 30 chromosomes (which were confirmed by cytogenetic analysis) were sown in the field at optimum sowing date (25th November) and were allowed to self-pollinate in order to develop monosomic and disomic lines for the D-genome of wheat to be used for further genetic analysis and also in the future wheat breeding programs (data not shown).

Cytological analysis

The cytogenetical examination of pollen mother cells of the tested parents and their progenies (F₁ pentaploids and F₂) were achieved according to Bhagyalakshmi *et al.*, (2008) in order to confirm their chromosome number and to study their meiotic behavior. Mean number of lost chromosomes in F₂ gametes were calculated according to Wang *et al.*, (2005).

Evaluating wheat genotypes for salinity tolerance

The tested parents and their F₁ hybrids (21 genotypes) were subjected to a laboratory experiment in order to evaluate their salinity tolerance. Seeds taken from the tested genotypes were disinfected by immersion in sodium hypochlorite solution (5 %) for five minutes, then washed three times with distilled water, and allowed to germinate in plastic dishes on filter papers soaked with distilled water for control and 100 mM NaCl solution for salinity stress (Datta *et al.*, 2009). The experiment was conducted with three replications in a growth chamber with 25°C under dark conditions for the first three days. Each replication of the two treatments (0 and 100 mM NaCl) contains 20 seeds for each genotype. Seedlings were harvested on the 12th day and separated from the remaining seeds. Germination percentage (%) and growth parameters at seedling stage including root length (cm), shoot length (cm), seedling fresh weight (g) and seedling dry weight (g) were then measured for each genotype.

The vigor index (VI) of each genotype was calculated following Abdul-Baki and Anderson (1973). Salt tolerance index (STI) of each genotype was calculated for seed germination (%) and seedling traits by the formula described by Goudarzi and Pakniyat (2008). However, based on mean STI values, the tested parents and their F₁ hybrids (21 genotypes) were classified into four categories, namely: 1) Highly salt tolerant (HST), STI= 80 to 100%, 2) Salt tolerant (ST), STI= 70 to < 80 %, 3) Moderately salt tolerant (MST), STI= 60 to < 70 % and 4) Salt sensitive (SS), STI= 50 to < 60 %. The genotypes were then ranked according to their mean STI following Ahmad *et al.*, (2013).

Statistical analyses

The crossability of each interspecific cross combination between durum and bread wheat genotypes was expressed as the percentage of pollinated florets giving embryo-containing caryopses. The data of the crossability of wheat genotypes were subjected to an analysis of variance (ANOVA) to test the significance of the difference between direct and reciprocal crosses of each interspecific cross combination. The differences between means were tested by Fisher's Least Significant Difference (LSD) at 0.05 and 0.01 probability levels. To

test for the significance of differences among the genotypes (G), environments (E) and the significance of G×E interaction for seed germination and seedling traits, data of the parents and their F₁'s were analyzed using a combined ANOVA across two environments (0 and 100 mM NaCl). The broad-sense heritability (h^2_B) of each trait was then calculated by using the expected value of variance and the formula described by Nyquist (1991).

RESULTS AND DISCUSSION

Interspecific hybridization and crossability

In the present study both of direct and reciprocal crosses between three hexaploid and three tetraploid wheat genotypes were made to produce eighteen F₁ pentaploid hybrids. All crosses successfully produced enough number of F₁ seeds irrespective of the cross direction. ANOVA for the crossability (%) between durum and bread wheat genotypes (Table 2) revealed high significant differences ($P < 0.01$) among the interspecific crosses and between direct and reciprocal crosses. On average, the direct crosses significantly ($P < 0.01$) showed higher crossability than reciprocal crosses (72.5 and 51.1%, respectively) (Table 3 and Figure 1). These differences in crossability were dependent on the parental genotypes and cross direction. Accordingly, when the tetraploid genotypes were used as maternal parents (direct crosses) the crossability ranged from 46.7 (P₃ × P₅) to 96.3 % (P₁ × P₅), however when the hexaploid genotypes were used as maternal parents (reciprocal crosses) the crossability ranged from 33.3 (P₃ × P₄) to 64.5% (P₁ × P₆). On average, using of tetraploid genotypes as maternal parents increased the crossability by 41.9 % as compared to using hexaploid genotypes as maternal parent. These findings are in accordance with other reports that the rate of crossability is high if tetraploid species are pollinated with the pollen grains of a hexaploid species (Bhagyalakshmi *et al.*, 2008; Naskidashvili *et al.*, 2012).

Table 2. ANOVA for the crossability (%) between durum and bread wheat genotypes.

Source of variation	d.f	SS	MS	F
Replicates	2	651.19	325.59	4.67*
Interspecific Crosses	17	14024.88	824.99	11.84**
Direct Crosses	8	4892.07	611.51	8.77**
Reciprocal Crosses	8	2865.57	358.20	5.14**
Direct vs Reciprocal	1	6267.24	6267.24	89.91**
Error	34	2369.91	69.70	

* and **: significant differences at 0.05 and 0.01 level of probability, respectively.

Table 3. Mean crossability (%) and differences between direct and reciprocal crosses.

Cross combination	Crossability (%)		Mean	Difference
	Direct	Reciprocal		
P ₁ × P ₄	66.2	46.6	56.4	19.6 **
P ₁ × P ₅	96.3	54.5	75.4	41.8 **
P ₁ × P ₆	76.3	64.5	70.4	11.8 NS
P ₂ × P ₄	77.7	56.4	67.1	21.3 **
P ₂ × P ₅	76.4	40.3	58.4	36.1 **
P ₂ × P ₆	61.5	45.2	53.4	16.3 *
P ₃ × P ₄	86.4	33.3	59.9	53.1 **
P ₃ × P ₅	46.7	55.0	50.9	8.3 NS
P ₃ × P ₆	64.7	63.8	64.3	0.9 NS
Average	72.5	51.1	61.8	21.4 **

LSD (0.05) = 13.9 , LSD (0.01) = 18.6

Direct cross: tetraploid as a female parent; Reciprocal cross: hexaploid as a female parent.* and **: significant differences between direct and reciprocal crosses at 0.05 and 0.01 level of probability, respectively. NS: nonsignificant differences.

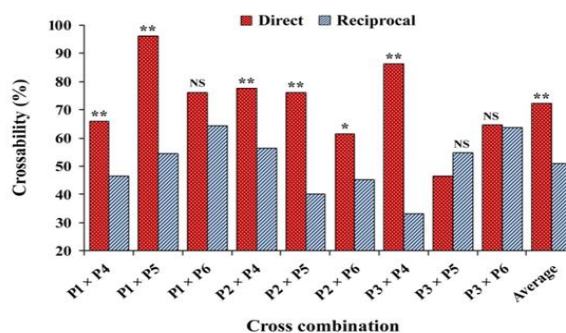


Fig. 1. The crossability (%) of direct and reciprocal crosses. Direct cross: tetraploid as a female parent; Reciprocal cross: hexaploid as a female parent.* and **: significant differences between direct and reciprocal crosses according to LSD test at 0.05 and 0.01 level of probability, respectively. NS: nonsignificant differences.

Meiotic behavior of pentaploid F₁ hybrids

All pentaploid plants for each cross were morphologically similar and successfully produced seeds. Also, they had 35 chromosomes consisting of 14 bivalents (A and B genomes) and 7 univalents (a single dose from D genome) at metaphase-I stage. However abnormal chromosomes behavior was observed in the later stages of meiosis due to the irregular segregation of D genome univalents. The chromatin bridges were observed in anaphase-I stage and lagging chromosomes were observed in telophase-I and telophase-II stages which leading to form the micronuclei structures in the tetrad stages in some cases (Figure 2).

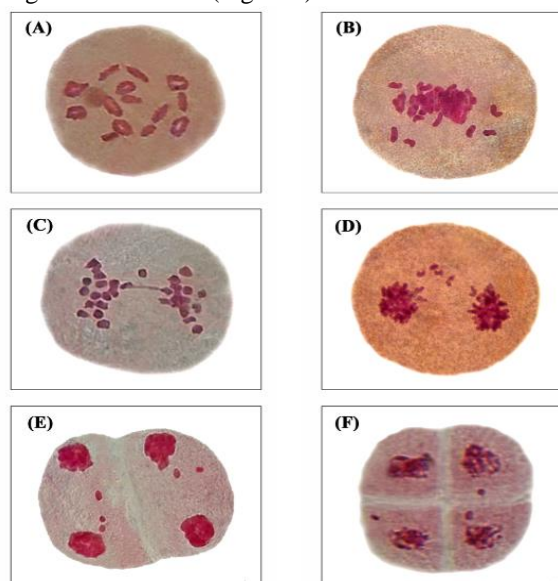


Fig. 2. Abnormal meiotic behavior in pentaploid F₁ hybrids: (A) diakinesis having 7 bivalents (genomes A and B) and 7 univalents (genome D); (B) some of D genome univalents are dispersed around the cell equatorial line at metaphase-I stage; (C) chromatin bridge at anaphase-I stage; (D) lagging chromosomes at telophase-I stage; (E) lagging chromosomes at telophase-II stage and (F) micronuclei structure at the tetrad stage.

Cytogenetic analysis and Morphology of F₂ plants

Cytogenetic analysis of F₂ plants revealed high variations in the percentages of plants with specific chromosome number within and among the F₂ populations tested (Table 4 and Figure 3). On average, the F₂

populations had chromosome number ranged from $2n = 31.96$ ($P_3 \times P_4$) to $2n = 33.71$ ($P_3 \times P_5$), indicating that each gamete lost 5.02 to 4.15 chromosomes at meiosis of the F_1 pentaploid plants, respectively. Notably, four crosses could produce at least one plant for each with 42 chromosomes, while no plants with 28 chromosomes were observed in any cross tested. Also, the Plants with chromosome numbers of 35, 37, 38, 40 and 41 were not recorded in some crosses. Generally, the plants with chromosomes number lower than 35 were more frequent than those with chromosomes number higher than 35. In these regards, there are several studies investigated the variation in frequency of chromosome number in the F_2 plants derived from F_1 pentaploid (Kihara, 1982; Wang *et al.*, 2005; Eberhard *et al.*, 2010; Martin *et al.*, 2011; Padmanaban *et al.*, 2018). In accordance with our results, they found that each F_2 population derived from F_1 pentaploid has its unique pattern regard to frequency distribution of plants with specific chromosomes number; however, they

suggested that the retention of D chromosomes in the F_2 plants is depending on the parents of the original cross. Moreover, it appears that some gametes with specific chromosome number may be superior and have higher chance for fertilization than others depending on their genetic background. Interestingly, the self-pollination of the derived F_2 plants with 42 chromosomes in the present study which having all wheat A, B and D chromosomes would produce stable bread wheat lines exploiting some genes from the durum wheat which can be used to improve bread wheat in the future. In addition, if these plants were backcrossed with the hexaploid parent, this could be very efficient for improving bread wheat via rapid introgression of desired genes from durum wheat. Furthermore, the other lines with lower chromosomes number can be used to improve the durum wheat via self-pollination or backcrossing with the tetraploid parent followed by selection of lines with $2n = 28$ chromosomes which allow to exploit desirable genes from the bread wheat.

Table 4. Frequency (%) of chromosomes distribution in F_2 populations.

F ₂ populations	Chromosome number														C	M
	29	30	31	32	33	34	35	36	37	38	39	40	41	42		
$P_1 \times P_4$	9.76	12.2	17.07	4.88	17.07	9.76	2.44	12.2	7.32	2.44	2.44	2.44	0	0	33.1	4.45
$P_2 \times P_4$	10	18	16	16	8	6	6	10	4	0	2	2	0	2	32.66	4.67
$P_3 \times P_4$	20.41	14.29	20.41	8.16	14.29	10.2	0	4.08	4.08	0	2.04	0	0	2.04	31.96	5.02
$P_1 \times P_6$	12.28	21.05	10.53	10.53	19.3	10.53	1.75	7.02	0	3.51	1.75	0	0	1.75	32.35	4.82
$P_2 \times P_6$	15.91	13.64	9.09	11.36	15.91	13.64	0	6.82	4.55	4.55	4.55	0	0	0	32.68	4.66
$P_3 \times P_5$	11.29	11.29	9.68	12.9	9.68	8.06	1.61	12.9	3.23	8.06	4.84	3.23	1.61	1.61	33.71	4.15
Average	13.27	15.08	13.8	10.64	14.04	9.7	2.95	8.84	4.63	4.64	2.94	2.55	1.61	1.85	32.74	4.63

C: Mean chromosome number in all F_2 plants, M: Mean number of lost chromosomes in gametes.

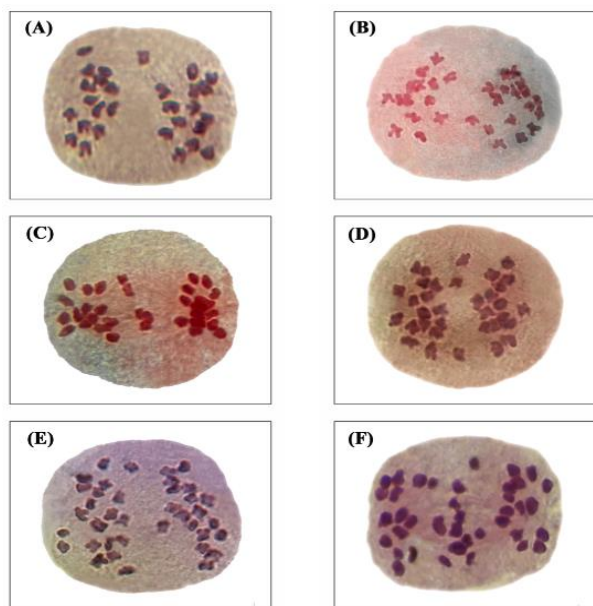


Fig. 3. Pollen mother cells (PMC) at anaphase-I stage in F_2 plants representing unequal segregations in cells with different chromosomes numbers: (A) $2n = 29$; (B) $2n = 31$; (C) $2n = 32$; (D) $2n = 34$; (E) $2n = 38$ and (F) $2n = 39$.

The F_2 progenies derived from the six interspecific crosses tested were varied morphologically; and these variations are due to their different chromosomes content. Some F_2 plants in each cross could not produce seeds and

were considered as sterile. However, the percentage of fertility in the F_2 populations ranged from 88.30 ($P_3 \times P_5$) to 98.41 % ($P_2 \times P_6$) as shown in Table 5. Similar findings were observed by Wang *et al.*, (2005) as they reported that this sterility is due to pollen grains sterility. Moreover, F_2 seeds of the tested populations showed high variability in the germination (%) in the field which ranged from 50 ($P_1 \times P_4$) to 78.30 % ($P_3 \times P_5$) (Table 5). In this regard, Prazak (2001) suggested that the low viability in some F_2 seeds may due to bad interrelation of the embryo and endosperm in developing seed; embryo development is interrelated with growing endosperm in the early stages of germination but later the embryo becomes self-sufficient. Interestingly, significant positive correlations were observed between chromosome number and plant height in F_2 populations of the crosses $P_1 \times P_4$ ($r = 0.37$; $P < 0.05$), $P_2 \times P_6$ ($r = 0.48$; $P < 0.01$) and $P_3 \times P_5$ ($r = 0.42$; $P < 0.01$). Significant positive correlations were also observed between chromosome number and spike length in F_2 populations of the crosses $P_1 \times P_4$ ($r = 0.39$; $P < 0.05$), $P_2 \times P_4$ ($r = 0.35$; $P < 0.05$) and $P_3 \times P_5$ ($r = 0.44$; $P < 0.01$). Overall F_2 populations, highly significant ($P < 0.01$) positive correlations were observed between chromosome number with plant height ($r = 0.23$) and spike length ($r = 0.27$) (Table 6). These findings are in accordance with those observed by Wang *et al.*, (2005). It seems that the wheat D genome has a potential positive effect on plant height and spike length, and these impacts might depend on the source of the D chromosomes.

Table 5. Percentages of seed germination and plant fertility in F_2 populations.

Cross	No. of Sown seeds	No. of Germinated seeds	Germination(%)	No. of Fertile plants	No. of Sterile plants	Fertile plants(%)
$P_1 \times P_4$	100	50	50.0	49	1	98.00
$P_2 \times P_4$	100	62	62.0	60	2	96.77
$P_3 \times P_4$	100	70	70.0	66	4	94.29
$P_1 \times P_6$	120	70	58.3	65	5	92.86
$P_2 \times P_6$	100	63	63.0	62	1	98.41
$P_3 \times P_5$	120	94	78.3	83	11	88.30

Table 6. Chromosome number, plant height(cm) and spike length (cm) of parental genotypes and F₂ plants, and the correlation(r) between the chromosome number with plant height and spike length in six F₂ populations.

Genotypes	Chromosome number			Plant height (cm)		Spike length (cm)	
	Min	Max	Mean	Mean	r	Mean	r
Parents	P ₁	28	28	28	93.0	8.5	
	P ₂	28	28	28	95.2	8.8	
	P ₃	28	28	28	92.5	9.0	
	P ₄	42	42	42	108.2	10.2	
	P ₅	42	42	42	122.5	20.0	
	P ₆	42	42	42	112.0	12.5	
F ₂ populations	P ₁ ×P ₄	29	40	33.1	100.2	10.0	0.37*
	P ₂ ×P ₄	29	42	32.7	94.8	10.1	0.35*
	P ₃ ×P ₄	29	42	31.9	98.6	9.0	-0.12
	P ₁ ×P ₆	29	42	32.3	114.5	11.0	0.16
	P ₂ ×P ₆	29	39	32.7	103.2	10.3	0.16
	P ₃ ×P ₅	29	42	33.7	102.7	13.6	0.44**
	Overall	29	42	32.7	102.8	11.2	0.27**

* and **: significant correlations at 0.05 and 0.01 level of probability, respectively.

Performance of genotypes under salinity stress

The combined ANOVA (Table 7) revealed high significant differences ($P < 0.01$) between control and salinity stress treatments as well as among the tested wheat genotypes for all studied traits. On average, salinity stress of 100 mM NaCl reduced germination percentage (GP), root length (RL), shoot length (SL), seedling fresh weight (FW), seedling dry weight (DW) and vigor index (VI) by 25.1, 28.9, 39.0, 37.2, 37.7 and 50.5%, respectively (Table 8 and Table 9). In accordance to our results, the reduction in germination percentage and different growth parameters of wheat seedlings was observed at a concentration of 100 mM NaCl (Oyiga *et al.*, 2016; Zou *et al.*, 2016; Hussain *et al.*, 2019). Moreover, Datta *et al.*, (2009) reported that the effects of salinity on germination rate, RL, SL, FW and DW of wheat seedlings are almost prominent from 100 mM NaCl onwards, and the effect of salinity was completely inhibitory at concentrations of 125 and 150mM NaCl. The reductions in seed germination and various seedling traits in different wheat genotypes under different levels of salinity have been widely reported in wheat (Hussain *et al.*, 2013; Kochak-Zadeh *et al.*, 2013; Guo *et*

al., 2015; Alom *et al.*, 2016; Bilkis *et al.*, 2016; Hasanuzzaman *et al.*, 2017). It has been reported that salinity stress is caused by the high accumulation of soluble salt in the soil and water; especially NaCl (Hussain *et al.*, 2019). Consequently, the higher concentration of soluble salts in the soil profile may cause physiological drought to the plant and reduction in the water uptake due to salt accumulation in the root zone (Munns, 2005). Higher salinity causes high osmotic stress and ion toxicity due to low water potential of the soil and excess Na⁺ accumulation within plant tissues which finally leading to numerous morphological, physiological, and biochemical deleterious effects on the plants (Hasanuzzaman *et al.*, 2017). The reduction in seed germination under salinity stress condition may be due to the loss of viability at higher salinity level, whereas the reduction in root and shoot development and elongation may be caused by one or more of the following factors: 1) toxic effects of the higher level of NaCl concentration 2) unbalanced nutrient uptake by the seedlings and 3) slowing down the water uptake of the plant (Datta *et al.*, 2009).

Table 7. Mean squares of the combined ANOVA and broad-sense heritability (h²B) of germination percentage (GP), root length (RL), shoot length (SL), seedling fresh weight (FW) and seedling dry weight (DW) under control (0 mM NaCl) and salinity stress (100 mM NaCl) environments.

Source of variation	d.f	Mean square				
		GP	RL	SL	FW	DW
Environments (E)	1	11071.9**	124.28**	536.31**	33636.3**	229.92**
Replicates within E	4	154.12*	2.87*	2.81	475.36**	5.26**
Genotypes (G)	20	560.62**	10.77**	28.65**	2243.80**	28.59**
Durum parents (D)	2	38.89	0.14	0.77	361.88*	2.81
Bread parents (B)	2	17.01	0.81	4.86	353.08*	3.82*
F ₁ hybrids	14	535.99**	13.39**	23.43*	1528.38**	22.34**
D vs. B vs. F ₁	2	1798.30**	13.04**	116.94**	11024.4**	122.92
D vs. B	1	2458.51**	8.51**	113.69**	5320.04**	4.82*
Parents vs. F ₁	1	1138.10**	17.57**	120.19**	16728.7**	241.02**
G × E interactions	20	152.92**	1.86*	2.36*	397.82**	22.34**
Pooled error	80	53.28	1.03	1.20	101.76	3.09
σ^2_G		67.95	1.49	4.38	307.66	12.32
σ^2_E		53.28	1.03	1.20	101.76	2.90
σ^2_{GE}		33.21	0.28	0.38	98.68	2.02
$h^2(B)$		0.44	0.53	0.73	0.61	0.71

*, **: significant differences at 0.05 and 0.01 probability, respectively. $h^2_B = \sigma^2_G / \sigma^2_P$, the phenotypic variance (σ^2_P) = $\sigma^2_G + \sigma^2_E + \sigma^2_{GE}$, where σ^2_G = the variance of genetic effect, σ^2_E = the environmental variance and σ^2_{GE} is the variance of G × E interactions.

Consistently, bread wheat genotypes showed higher GP than durum wheat genotypes under control (0 mM NaCl) and salinity stress (100 mM NaCl) treatments, with an average of 86.1 and 80.6% under control and 72.5 and 45.0% under salinity stress, respectively. On average, bread wheat genotypes had longer shoots and roots as well as higher FW than durum wheat genotypes under control and salinity stress treatments (Tables 8 and 9). However, durum wheat genotypes had higher DW under control. Distinctly, all bread wheat genotypes were more vigorous than durum wheat under both treatments (Table 9). Obviously, salinity stress affected parental durum wheat

genotypes and their tetraploid hybrids higher than its effect on parental hexaploid wheat genotypes and their hexaploid hybrids for all the studied traits. However, in general, pentaploid F₁ hybrids showed moderate reductions for all the traits comparing to their respective tetraploid and hexaploid parents (Table 8 and Table 9). It has been reported that bread wheat is known to possess higher salt tolerance compared with durum wheat (Munns *et al.*, 2000; Munns *et al.*, 2006; Munns and Tester 2008). Higher salinity tolerance in bread wheat has been mainly attributed to the better ability of bread wheat to exclude Na⁺ from uptake (Colmer *et al.*, 2006; Cuin *et al.*, 2010, Munns *et*

al., 2012; Wu et al., 2014). As a result, bread wheat accumulates less Na⁺ in the shoot, relative to durum wheat (Wu et al., 2014), and thus maintains a higher K⁺/Na⁺ ratio in leaves (Munns et al., 2003; Lindsay et al., 2004). In this

regard the ability to maintain low Na⁺ and high K⁺ in leaves was found to be associated with salt tolerance within cultivated wheat species (Munns and James 2003; Poustini and Siosemardeh 2004; Colmer et al., 2006).

Table 8. Means of germination percentage (GP), root length (RL) and shoot length (SL) of parental wheat genotypes and their tetraploid, hexaploid and pentaploid F₁ hybrids under control (C) and salinity stress (S) treatments.

Genotypes	GP (%)			RL (cm)			SL (cm)				
	C	S	Red %	C	S	Red (%)	C	S	Red (%)		
P ₁	Sohag-3	80.0	40.0	50.0	8.2	4.0	51.6	10.9	5.5	49.5	
P ₂	BeniSuef-5	80.0	46.7	41.7	7.2	4.7	34.8	11.9	5.2	56.1	
P ₃	Svevo	81.7	48.3	40.8	6.8	4.8	29.5	11.4	6.5	43.1	
Mean		80.6	45.0	44.2	7.4	4.5	38.6	11.4	5.7	49.6	
P ₄	Sakha-8	90.0	72.5	19.4	7.2	6.1	15.5	13.9	10.6	24.2	
P ₅	Line-6	85.0	71.7	15.7	7.2	6.3	12.7	14.7	11.2	24.2	
P ₆	Misr-2	83.3	73.3	12.0	8.1	6.6	17.8	13.1	9.2	29.9	
Mean		86.1	72.5	15.7	7.5	6.3	15.4	13.9	10.3	26.1	
F ₁ hybrids	Tetraploid	P ₁ × P ₂	80.0	55.0	31.3	6.7	4.2	37.2	9.1	4.9	45.7
		P ₁ × P ₃	78.3	48.3	38.3	7.0	3.9	45.3	11.1	5.2	53.4
		P ₂ × P ₃	85.0	48.3	43.1	7.3	3.4	54.0	11.3	5.1	55.0
		Mean	81.1	50.6	37.6	7.0	3.8	45.5	10.5	5.1	51.4
	Hexaploid	P ₄ × P ₅	83.3	66.7	20.0	8.0	6.8	15.4	12.3	10.1	17.8
		P ₄ × P ₆	85.0	71.7	15.7	9.5	7.4	21.8	13.1	10.1	23.0
		P ₅ × P ₆	81.7	66.7	18.4	8.9	7.6	14.7	13.1	10.2	22.5
		Mean	83.3	68.3	18.0	8.8	7.3	17.3	12.8	10.1	21.1
	Pentaploid	P ₁ × P ₄	65.0	40.0	38.5	6.7	4.5	33.2	10.0	5.1	48.7
		P ₁ × P ₅	55.0	38.3	30.3	6.6	4.8	28.0	10.9	6.0	44.8
		P ₁ × P ₆	50.0	41.7	16.7	8.6	4.4	48.7	11.2	6.0	46.4
		P ₂ × P ₄	70.0	61.7	11.9	5.3	4.2	19.9	9.4	6.6	29.6
		P ₂ × P ₅	68.3	60.0	12.2	5.8	5.4	7.9	10.0	6.3	36.8
		P ₂ × P ₆	68.3	58.3	14.6	4.5	3.3	26.3	7.5	3.8	49.3
		P ₃ × P ₄	73.3	61.7	15.9	5.2	3.1	41.2	7.1	4.6	36.0
		P ₃ × P ₅	75.0	60.0	20.0	4.4	3.4	23.6	7.4	5.2	29.6
	P ₃ × P ₆	75.0	60.0	20.0	4.4	3.2	28.6	8.6	4.0	53.7	
	Mean	66.7	53.5	20.0	5.7	4.0	28.6	9.1	5.3	41.6	
Overall Mean		75.9	56.7	25.1	6.8	4.8	28.9	10.8	6.7	39.0	
LSD (0.05)		7.1	8.2	-	1.0	1.0	-	1.5	1.7	-	
LSD (0.01)		9.8	11.2	-	1.4	1.3	-	2.1	2.3	-	
CV (%)		13.4	20.5	-	21.4	29.3	-	19.8	35.5	-	

Red (%): Reduction percentage resulting by salinity stress (100 mM NaCl).

Table 9. Means of seedling fresh weight (FW), seedling dry weight (DW), and vigor index (VI) of parental wheat genotypes and their tetraploid, hexaploid and pentaploid F₁ hybrids under control (C) and salinity stress (S) treatments.

Genotypes	FW (mg)			DW (mg)			VI				
	C	S	Red (%)	C	S	Red (%)	C	S	Red (%)		
P ₁	Sohag-3	107.6	44.0	59.1	17.3	11.4	34.5	1529	379	75.2	
P ₂	BeniSuef-5	117.3	58.5	50.1	17.8	7.7	56.5	1525	462	69.7	
P ₃	Svevo	96.2	50.7	47.3	15.9	8.3	47.9	1484	544	63.3	
Mean		107.0	51.0	52.2	17.0	9.1	46.3	1513	462	69.4	
P ₄	Sakha-8	104.5	89.2	14.7	16.1	15.2	5.3	1905	1209	36.5	
P ₅	Line-6	119.0	104.7	12.0	16.2	12.4	23.1	1861	1249	32.9	
P ₆	Misr-2	117.7	85.0	27.7	15.2	10.7	29.2	1764	1160	34.2	
Mean		113.7	93.0	18.1	15.8	12.8	19.2	1843	1206	34.6	
F ₁ hybrids	Tetraploid	P ₁ × P ₂	106.4	48.9	54.0	15.4	7.9	48.6	1262	502	60.2
		P ₁ × P ₃	97.2	42.6	56.2	16.5	7.8	52.5	1418	435	69.3
		P ₂ × P ₃	95.5	62.9	34.2	14.0	7.1	49.6	1585	409	74.2
		Mean	99.7	51.4	48.1	15.3	7.6	50.2	1422	449	67.9
	Hexaploid	P ₄ × P ₅	100.7	81.4	19.1	14.6	10.5	27.8	1693	1126	33.5
		P ₄ × P ₆	96.4	85.9	11.0	16.0	11.3	29.5	1918	1253	34.7
		P ₅ × P ₆	98.5	72.7	26.2	14.9	10.4	30.0	1795	1182	34.2
		Mean	98.6	80.0	18.8	15.2	10.7	29.1	1802	1187	34.1
	Pentaploid	P ₁ × P ₄	64.8	52.5	19.0	5.6	4.8	14.0	1082	383	64.6
		P ₁ × P ₅	84.8	51.7	39.0	8.2	5.0	38.7	961	412	57.1
		P ₁ × P ₆	76.3	41.8	45.2	8.7	3.4	60.4	991	435	56.2
		P ₂ × P ₄	68.2	46.4	31.9	9.1	5.6	38.4	1025	667	34.9
		P ₂ × P ₅	66.7	40.0	40.0	8.5	5.2	38.4	1081	701	35.2
		P ₂ × P ₆	74.2	30.0	59.6	8.3	4.8	42.3	817	414	49.3
		P ₃ × P ₄	60.0	34.7	42.2	6.6	4.4	33.3	905	470	48.1
		P ₃ × P ₅	53.2	30.1	43.5	5.4	3.3	39.4	883	513	41.9
	P ₃ × P ₆	70.4	35.7	49.3	7.5	3.6	51.5	981	431	56.1	
	Mean	68.7	40.3	41.1	7.5	4.5	39.6	970	492	49.2	
Overall Mean		89.3	56.6	37.2	12.3	7.7	37.7	1355	683	50.5	
LSD (0.05)		14.3	15.2	-	3.1	2.4	-	268	242	-	
LSD (0.01)		19.6	20.9	-	4.2	3.3	-	368	332	-	
CV (%)		22.7	38.2	-	35.6	44.3	-	28.1	50.3	-	

Red (%): Reduction percentage resulting by salinity stress (100 mM NaCl).

Moderate to high broad-sense heritability estimates were found for GP (0.44), RL (0.53), SL (0.73), FW (0.61) and DW (0.71), indicating the presence of considerable genetic variances (Table 7). Similar results were observed for seed germination and seedling traits under different levels of salinity in wheat (Ali *et al.*, 2007; Shahzad *et al.*, 2012; Al-Ashkar and El-Kafafi 2014; Oyiga *et al.*, 2016; Dadshani *et al.*, 2019; Al-Ashkar *et al.*, 2020).

Salt tolerance index (STI)

High variations of STI values measured based on GP, RL, SL, FW and DW were observed between the parents and their F₁ hybrids (Table 10). The mean STI ranged from 0.51 (P₁ and P₁×P₃) to 0.84 (P₄). Highly significant (P<0.01) and strong positive correlations were observed between mean STI with STI of GP (r=0.71), RL (r=0.85), SL (r=0.92), FW (r=0.84) and DW (r=0.78). Therefore, the genotypes were then ranked based on their mean STI. Constantly, bread wheat genotypes and their hexaploid hybrids had higher STI than durum wheat genotypes and their tetraploid hybrids. However, moderate estimates of mean STI were observed for pentaploid F₁ hybrids which ranged from 0.57 (P₁×P₆) to 0.74 (P₂×P₄). Based on mean STI estimates, the tested genotypes were divided into four categories (Table 10). As mentioned earlier, the pentaploid hybrid strategy is an effective tool to transfer desirable traits and genes from tetraploid wheat into hexaploid wheat and vice versa. Transferring desirable traits or genes became more easy and faster when the genes of the concern are located on A and/or B genomes (Martin *et al.*, 2013); because these genomes are present in both of tetraploid and hexaploid wheat. However, transferring genes from D genome of the hexaploid wheat into durum wheat became more difficult because the absence of D

genome in the durum wheat as in the case of salinity tolerance (Han *et al.*, 2014); the *Kna1* gene that confers salinity tolerance in hexaploid wheat is located on the long arm of chromosome number 4 in D genome (Dubcovsky *et al.*, 1996). In this regard, Han *et al.*, (2014) and Han *et al.*, (2016) demonstrated the successful introgression of salt and aluminum tolerance genes from D genome of bread wheat into B genome in durum wheat using pairing homeologous (*ph1c*) mutation strategy; this strategy allow generating recombination between chromosomes 4B and 4D. However, this method requires specific 4D (4B) substitution line of durum wheat to start the breeding program which makes it more complex. Surprisingly, several reports informed the spontaneous introgression of some D segments into A or B genomes in the progenies of pentaploid hybrids (Eberhard *et al.*, 2010; Deng *et al.*, 2018; Othmeni *et al.*, 2019); the number and frequency of the introgressed segments were dependent on the genetic background of the hexaploid and tetraploid parents. In the present study, most of the pentaploid F₁ hybrids produced were more salt tolerance than their tetraploid parents suggesting that salinity tolerance genes of the bread wheat genotypes tested were transmitted to their pentaploid F₁ hybrids. Moreover, the degree of tolerance in the pentaploid hybrids depended on the genetic background of their hexaploid parents. Hence, introgression of some D genome segments into durum wheat could be occurs. Therefore, backcrossing of the superior pentaploid hybrids which were created in the present study with their tetraploid parents and evaluation of their subsequent progenies for salt tolerance and chromosome content could provide an effective tool to improve salt tolerance in durum wheat.

Table 10. Stress tolerance index (STI) estimates of parental genotypes and their F₁ hybrids based on germination percentage (GP), root length (RL), shoot length (SL), seedling fresh weight (FW) and seedling dry weight (DW).

Genotypes	STI					Mean STI	Rank	Category		
	GP	RL	SL	FW	DW					
P ₁ Sohag-3	0.50	0.48	0.51	0.41	0.66	0.51	17	SS		
P ₂ BeniSuef-5	0.58	0.65	0.44	0.50	0.44	0.52	16	SS		
P ₃ Svevo	0.59	0.71	0.57	0.53	0.52	0.58	13	SS		
P ₄ Sakha-8	0.81	0.84	0.76	0.85	0.95	0.84	1	HST		
P ₅ Line-6	0.84	0.87	0.76	0.88	0.77	0.82	2	HST		
P ₆ Misr-2	0.88	0.82	0.70	0.72	0.71	0.77	5	ST		
F ₁ hybrids	Tetraploid	P ₁ ×P ₂	0.69	0.63	0.54	0.46	0.51	14	SS	
		P ₁ ×P ₃	0.62	0.55	0.47	0.44	0.47	0.51	17	SS
		P ₂ ×P ₃	0.57	0.46	0.45	0.66	0.50	0.53	15	SS
	Hexaploid	P ₄ ×P ₅	0.80	0.85	0.82	0.81	0.72	0.80	3	HST
		P ₄ ×P ₆	0.84	0.78	0.77	0.89	0.70	0.80	3	HST
		P ₅ ×P ₆	0.82	0.85	0.77	0.74	0.70	0.78	4	ST
	Pentaploid	P ₁ ×P ₄	0.62	0.67	0.51	0.81	0.86	0.69	8	MST
		P ₁ ×P ₅	0.70	0.72	0.55	0.61	0.61	0.64	10	MST
		P ₁ ×P ₆	0.83	0.51	0.54	0.55	0.40	0.57	14	SS
		P ₂ ×P ₄	0.88	0.80	0.70	0.68	0.62	0.74	6	ST
		P ₂ ×P ₅	0.88	0.92	0.63	0.60	0.62	0.73	7	ST
		P ₂ ×P ₆	0.85	0.74	0.51	0.40	0.58	0.62	11	MST
		P ₃ ×P ₄	0.84	0.59	0.64	0.58	0.67	0.66	9	MST
		P ₃ ×P ₅	0.80	0.76	0.70	0.56	0.61	0.69	8	MST
P ₃ ×P ₆	0.80	0.71	0.46	0.51	0.48	0.59	12	SS		

Ranking wheat genotypes was performed based on mean STI. HST: highly salt tolerant (STI= 0.80 to 1.0), ST: salt tolerant (STI= 0.70 to < 0.80), MST: moderately salt tolerant (STI= 0.60 to < 0.70), SS: salt sensitive (STI= 0.50 to < 0.60).

CONCLUSION

The crossability percentage was high when the tetraploid wheat species were used as maternal parents. The concentration of 100 mM NaCl adversely affected on seed germination and seedling growth parameters of durum and bread wheat. Durum wheat and their tetraploid hybrids were more sensitive to salinity stress as compared to bread wheat and their hexaploid hybrids. However, in general, pentaploid F₁ hybrids showed moderate sensitivity to salinity comparing to their tetraploid and hexaploid parents. The pentaploid hybrid strategy used in the present study could be an effective tool to transfer desirable genes and traits between tetraploid and hexaploid wheat species.

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تقييم القابلية للتجهين بين الأقماع الرباعية والسداسية وتقييم الهجن الناتجة منها لتحمل الملوحة

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استهدف هذا البحث دراسة الاختلافات في قابلية التجهين بين ثلاث طرز وراثية من قمح الديورم الرباعي وثلاثة طرز وراثية من قمح الخبز السداسي، وكذلك دراسة اعداد الكروموسومات وسلوكها أثناء الإقسام الميوزي لهجن الجيل الأول الخماسية ونباتات الجيل الثاني الناتجة منها. تم أيضا تقييم تحمل الملوحة للطرز الأبوية وهجن الجيل الأول في مرحلة الإنبات وطور البادرة باستخدام تركيزات صفر و 100 ملي مول من كلوريد الصوديوم. أظهرت النتائج اختلافات معنوية جدا في النسبة المئوية لقابلية التجهين بين الأباء المستخدمة وكذلك بين الهجن والهجن العكسية. كانت النسبة المئوية للقابلية للتجهين أعلى عند استخدام الأباء الرباعية كأمهات (الهجن المباشرة). احتوت هجن الجيل الأول الخماسية على 35 كروموسوم وأظهرت الكروموسومات سلوكاً ميوزياً شاداً في المراحل المختلفة للإقسام الميوزي. أظهر التحليل الوراثي الخلوي لنباتات الجيل الثاني درجة عالية من الاختلافات في أعداد الكروموسومات بين العشار المدروسة وكذلك بين أفراد العشيرة الواحدة، مع تسجيل بعض النباتات المحتوية على 42 كروموسوم. أثر إجهاد الملوحة على الطرز الأبوية الرباعية وكذلك الهجن الرباعية الناتجة منها بدرجة أكبر من تأثيره على الطرز الأبوية السداسية والهجن السداسية الناتجة منها لجميع الصفات المدروسة. أما بالنسبة لهجن الجيل الأول الخماسية فقد كانت أقل تحملاً للملوحة مقارنة بأبائها السداسية إلا أنها كانت أكثر تحملاً للملوحة مقارنة بأبائها الرباعية، مما يشير إلى أن جينات تحمل الملوحة الخاصة بطرز قمح الخبز الأبوية قد انتقلت إلى هجن الجيل الأول الخماسية الناتجة منها. هذا ويمكن اعتبار إستراتيجية إنتاج الهجن الخماسية المستخدمة في الدراسة الحالية كأداة فعالة لنقل جينات وصفات مرغوبة بين أنواع القمح الرباعية والسداسية.