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## Inter Specific Crosses and Marker Assisted Selection for Improving the Nutritional Value of Egyptian Wheat Cultivars

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### ABSTRACT

Grain Protein Content (GPC) of wheat is important for improved nutritional value and is also one of the major factors affecting bread making and pasta quality. This study was an attempt to improve grain protein content in local wheat cultivars by transferring the Gpc-B1 allele from wild accessions of *Triticum turgidum* ssp. *dicoccoides* to Egyptian cultivars belonging to two wheat species, i.e., *Triticum aestivum* (Giza 164 and Sakha 69) and *Triticum durum* (Beni suif 5 and Sohag 3) using interspecific hybridization. The genetic variability among the studied genotypes (5 *T. turgidum* ssp. *dicoccoides*+2 *T. aestivum*+2 *Triticum durum*) was assessed using seven agronomic characters, grain protein (GPC), iron (GFeC) and zinc (GZnC) contents. Marker assisted selection using the Xuhw 89 marker was applied to confirm the presence of the Gpc-B1 gene in the wild *dicoccoides* parental genotypes and the F<sub>1</sub> hybrids. Nine out of the twelve hybrids revealed the Xuhw 89 allele of the *dicoccoides* (122 bp) and showed significant increase in the values of grain protein in addition to one or the two micronutrients (Fe and Zn) compared to the corresponding local cultivars. These values ranged from 88-147, 9-27 and 7-25 mg kg<sup>-1</sup>, respectively in the hybrids. While, they ranged from 72-97, 8-11 and 7-10 mg kg<sup>-1</sup>, respectively in the local cultivars. The results revealed that a weak negative correlation exists between grain yield and grain protein content. Nevertheless, some crosses (Giza 164×Irbid, Rachaya×Giza 164, Sakha 69×Haifa and Sohag 3×Haifa) exhibited an increase in grain protein content without affecting the grain yield.

**Key words:** Wheat species, grain protein content, Gpc-B1 gene, micronutrients, marker assisted selection (MAS)

### INTRODUCTION

Wheat is one of the most important crops worldwide. Together with rice and maize, wheat provides more than 60% of the calories and proteins for human nutrition (Gill *et al.* 2004). Wheat belongs to family *Poaceae* Tribe: *Triticeae*. The vast majority of the cultivated wheat grown worldwide is of two types: Hexaploid *Triticum aestivum* var. *aestivum* (genome constitution AABBDD) called bread wheat and tetraploid *Triticum turgidum* L., var. *durum* (genome constitution AABB) called durum wheat or pasta wheat. However, other varieties of these two species have been cultivated historically and are still grown in small amount in some parts of the world, in addition to other primitive diploid and tetraploid species. Further, a number of wild tetraploid species related to the progenitors of the A, B and D genomes of the cultivated wheats and

wild *T. turgidum* subsp. *dicoccoides* also occur, providing a reservoir of valuable variations for plant breeding (Heun *et al.*, 1997; Shewry, 2009). In Egypt wheat is the main winter cereal crop used as staple food for urban and rural societies and the major source of straw for animal feeding. Plant breeders have historically made major contribution to improve wheat quality and quantity to satisfy human needs.

Grain protein content (GPC) of wheat is important for improved nutritional value and is also one of the major factors affecting bread making and pasta quality (Finney *et al.*, 1987; Deckard *et al.*, 1996; Khan *et al.*, 2000). In bread wheat, the existence of a linear relationship between protein content and loaf volume has been reported by Finney and Barmore (1948).

Similarly, in durum wheat high protein content and strong gluten are major factors for producing pasta with superior cooking properties, better cooking firmness and tolerance to overcooking (D'Egidio *et al.*, 1990; Marchylo *et al.*, 1998). In spite of its importance, progress in breeding for high GPC has been slow and difficult. The limitation is that genetic variation for protein content is small compared with variation due to differences in growing environments. Also, there is a strong negative correlation between GPC and grain yield; cultivars with high GPC tend to be low yields (Blanco *et al.*, 2002, 2012). A promising source of high GPC was detected in a survey of wild tetraploid wheat populations of *Triticum turgidum* subsp. *dicoccoides* (Korn) (Avivi, 1978; Distelfeld *et al.*, 2004). This wild tetraploid wheat is carrying the allele Gpc-B1 which is also associated with increased mineral (iron and zinc) concentration in grain (Cakmak *et al.*, 2004). Therefore, enhancing the nutritional quality of wheat could be addressed through the introgression of the functional gene from the wild species into the cultivated durum and bread wheat (Gerechter-Amitai and Grama, 1977; Avivi, 1978; Grama *et al.*, 1984; Xu *et al.* 2011).

Marker assisted selection (MAS) is an efficient tool to facilitate tracing of introgressed genes (Slikova *et al.*, 2003; Khiabani *et al.*, 2009). MAS is a process whereby a marker is used for indirect selection of a genetic determinant of a trait of interest. A quantitative trait locus (QTL) for high GPC was detected in the short arm of the chromosome 6B from *Triticum turgidum* subsp. *dicoccoides* accession (DIC) (Khan *et al.*, 2000; Distelfeld *et al.*, 2006b). This quantitative trait locus was mapped as a single Mendelian locus designated Gpc-B1. A physical map of approximately 250 Kb of the Gpc-B1 region was developed using bacterial artificial chromosome library. Several DNA PCR markers flanking the Gpc-B1 locus were developed (Distelfeld *et al.*, 2004; 2006a). Efforts to improve GPC without selection for low yield can be accelerated by identification of the genes that affect GPC and the direct selection of the positive alleles with molecular markers (Distelfeld *et al.*, 2006b).

Therefore, the present study was conducted to address the following objectives: (1) To evaluate yield related characters, in addition to the protein, iron and zinc contents of four Egyptian wheat cultivars including two hexaploid and two tetraploid genotypes as well as five wild tetraploid wheat genotypes; (2) To attempt to transfer the Gpc-B1 allele, coding for high GPC from the wild wheat genotypes to the Egyptian wheat cultivars (hexaploid and tetraploid) via cross hybridization; (3) To assess the introgression of the Gpc-B1 gene in the genome of the F<sub>1</sub> hybrids using the marker assisted selection approach.

## **MATERIALS AND METHODS**

**Plant material:** Two hexaploid bread wheat (*Triticum aestivum* L.) varieties, two tetraploid durum (*Triticum turgidum* subsp. *durum*) and five wild emmer tetraploid wheat accessions were used in this investigation. The Egyptian hexaploid varieties (Giza 164 and Sakha 69) and

Table 1: Crosses established between the two hexaploid and the two tetraploid wheat genotypes and the five wild wheat genotypes

Cross	Cross name
P <sub>6</sub> ×P <sub>1</sub>	Giza 164×Haifa
P <sub>6</sub> ×P <sub>2</sub>	Giza 164×Irbid
P <sub>6</sub> ×P <sub>3</sub>	Giza 164×Sweida
P <sub>1</sub> ×P <sub>6</sub>	Haifa×Giza 164
P <sub>4</sub> ×P <sub>6</sub>	Rachaya×Giza 164
P <sub>7</sub> ×P <sub>1</sub>	Sakha 69×Haifa
P <sub>7</sub> ×P <sub>2</sub>	Sakha 69×Irbid
P <sub>7</sub> ×P <sub>3</sub>	Sakha 69×Sweida
P <sub>6</sub> ×P <sub>7</sub>	Baalbak×Sakha 69
P <sub>8</sub> ×P <sub>1</sub>	Beni-suif 5X Haifa
P <sub>9</sub> ×P <sub>1</sub>	Sohag 3×Haifa
P <sub>4</sub> ×P <sub>9</sub>	Rachaya×Sohag 3

tetraploid durum varieties (Beni-suif 5 and Sohag 3) were kindly provided by the Division of National Wheat Research Program, Agricultural Research Center, Giza, Egypt. While, the five wild emmer tetraploid wheat (Haifa, Irbid, Sweida, Rachaya and Baalbak) were provided by the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. Two wheat varieties: One hexaploid (Yecora Rojo) and one tetraploid (UC 1113+GPC) containing the Gpc-B1 locus were kindly provided by Prof. Jorge Dubcovsky, University of California, Davis, USA and used as control in the molecular analysis. The pedigree, origin and source of the wheat genotypes were as described in Hussein *et al.* (2012).

**Interspecific hybridization experiments:** Twelve crosses were developed between five selected accessions of the tetraploid wild species on one hand and the two hexaploid or tetraploid Egyptian wheat cultivars on the other hand as shown in Table 1.

The obtained hybrid grains were cultivated in the next season in order to obtain the F<sub>1</sub> plants and grains. Data were recorded for seven agronomic characters, grain protein content (GPC), grain iron content (GFeC) and grain zinc content (GZnC) on 15 individual plants (5 plants×3 replicates) from each of the parental genotypes and F<sub>1</sub> plants. The studied characters were: plant height (PH), No. of Tillers plant<sup>-1</sup> (NT), Main Spike Length (MSL), No. of spikelets spike<sup>-1</sup> (NS), No. of g/main spike(GS), grain index (100 grains weight, GI), Plant yield (Grain yield plant<sup>-1</sup>, PY), Grain protein content (GPC), Grain iron content (GFeC), Grain zinc content (GZnC).

**Statistical analysis:** Data of all characters were subjected to statistical analysis of a randomized complete blocks design. Sample means were compared using Duncan's New Multiple Range Test (Gomez and Gomez, 1976).

Analysis of variance of Randomized complete block design (RCBD) was performed also to test the significance of differences among means of the nine parents and their twelve crosses according to Snedecor and Cochran (1968). Differences between means of entries were tested for significance against LSD values.

**Heterosis:** Heterosis was expressed as the deviation of F1 generation from the Mid-Parent (MP) or High Parent (HP) average values as follows: Heterosis over mid-parent (MP)%:

Table 2: Xuhw 89 primer sequence for detection of the Gpc-B1 gene

Gpc-B1 primers		
Primer name	Sequence (5'-3')	Fragment size (bp)
Xuhw 89	BF 5'- TCT CCA AGA GGG GAG AGA CA -3' R5'-TTC CTC TAC CCA TGA ATC TAG CA -3'	122/126

(F1-MP)/MP×100 Heterosis over high-parent (HP)%: (F1-HP)/HP×100. To test the significance of the above estimate of heterosis, the variance of heterosis deviation was calculated as a linear function of following variances according to Wynne *et al.* (1970).

**Correlation studies:** Correlation coefficients between plant yield, grain protein content, grain iron content and grain zinc content for *T.aestivum* crosses and *T. durum* crosses and nine parents were computed according to Strickberger (1968) as follows:

$$R = \frac{\sum xy - (\sum x \sum y / N)}{\sqrt{\{\sum x^2 (\sum x)^2 / N\} \{\sum y^2 (\sum y)^2 / N\}}}$$

where, r = correlation,  $\sum xy$  = sum of varieties of both characters,  $\sum x$  and  $\sum y$  = sum of individual varieties, N = No. of varieties.

**Quantification of protein content:** Total content of protein was measured in wheat grains according to Bremner (1965).

**Quantification of iron and zinc content:** Total content of Zn and Fe was determined in wheat g by Atomic Absorption-Perkin -Elmer 1100B apparatus in the central labs of the National Research Center according to the method described by Chapman and Pratt (1961).

**DNA isolation:** Genomic DNA was extracted from the nine parental wheat genotypes and their F<sub>1s</sub> according to Dellaporta *et al.* (1983).

**PCR analysis for the detection of the Gpc-B1 gene:** The PCR based marker locus (Xuhw 89) was investigated using specific marker (Table 2) to detect the Gpc-B1 gene in the nine wheat genotypes. The Xuhw 89 is an indel marker located very close to the Gpc-6B1 gene and reveals a 4 bp deletion linked to the Gpc-6B1 gene in the genotype with high GPC (Distelfeld *et al.*, 2006a,b). Therefore, the expected size of the amplified product is 122 bp in the genotypes with high GPC while it is 126 bp for the genotypes with low GPC.

The amplification reaction was carried out in 25 µL total volume containing 1x PCR buffer, 1.5 mM MgCl<sub>2</sub>, 2 mM dNTPs, 2.5 U Taq DNA polymerase (all reagents from Promega Corp., USA), 10 mM primer and 25 ng template DNA, in a Biometra thermal cycler (Biomedizinische Analytik GmbH). The amplification program was comprised of a denaturation step at 94°C for 5 min, a No. of 37 cycles [94C/30, 61C/30 and 72°C/45sec] then a final extension step at 72°C for 5 min.

The products were analyzed using the Protein Cell System from BIO-RAD on 6% (w/v) non-denaturing polyacrylamide gel. PCR products were visualized on UV light and photographed using a gel documentation system (BIO-RAD).

**RESULTS AND DISCUSSION**

**Evaluation of the agronomic characters, grain protein content (GPC), grain iron content (GFeC) and grain zinc content (GZnC) in the parental genotypes:** As shown in Table 3, the results revealed that the wild accessions mostly exhibited low values of yield component characters such as main spike length, No. of spikelets per main spike, No. of grains per main spike, grain index and plant yield but they showed highly significant higher values from local cultivars in grain protein content, grain iron content, grain zinc content, plant height (with exception of Giza 164) and number of tillers. In this respect, Chee *et al.* (2001) evaluated the effect of the quantitative trait locus (QGpc.ndsu.6Bb) on GPC, grain yield and other agronomic traits in an adapted durum wheat background. They reported a weak negative correlation between yield and GPC for the LDN(DIC-6B) substitution line or genotypes derived from it. Bhutta (2006) investigated the association of some agronomic traits among wheat lines and their direct and indirect influence on the grain yield of wheat. The author's results revealed that the correlation and path analysis of grain yield and its components in promising wheat lines was positive and strongly correlated to No. of tillers and No. of spikes plant<sup>-1</sup>. Also grain yield was negatively correlated to the No. of florets spike<sup>-1</sup>. Khodarahmi *et al.* (2010) assessed the effects of plant breeding on yield and other characteristics of wheat cultivars. Their results showed that plant breeding has increased the grain yield through the decrease of plant height and the increase of harvest index. Number of

Table 3: Means of seven agronomic characters and GPC, Iron and zinc percent of the nine wheat genotypes and their hybrids

Parameters	PH	NT	MSL	NS	NG	GI	PY	GPC	GFeC	GZnC	Xuhw 89
Haifa (P <sub>1</sub> )	110 <sup>EF</sup>	30 <sup>BCD</sup>	6.5 <sup>J</sup>	11 <sup>E</sup>	23 <sup>LM</sup>	2.4 <sup>F</sup>	9.4 <sup>JKL</sup>	109 <sup>H</sup>	13 <sup>EF</sup>	15 <sup>E</sup>	122 bp
Irbid (P <sub>2</sub> )	100 <sup>GH</sup>	26 <sup>CDEF</sup>	6.7 <sup>HI</sup>	15 <sup>CDE</sup>	26 <sup>KL</sup>	2.5 <sup>F</sup>	8.3 <sup>KL</sup>	131 <sup>E</sup>	14 <sup>E</sup>	16 <sup>DE</sup>	122 bp
Sweida (P <sub>3</sub> )	100 <sup>GH</sup>	31 <sup>BC</sup>	5.7 <sup>I</sup>	13 <sup>DE</sup>	34 <sup>J</sup>	1.9 <sup>F</sup>	7.9 <sup>L</sup>	141 <sup>C</sup>	16 <sup>D</sup>	15 <sup>E</sup>	122 bp
Rachaya (P <sub>4</sub> )	100 <sup>GH</sup>	25 <sup>CDEF</sup>	6.0 <sup>J</sup>	19 <sup>ABC</sup>	28 <sup>JKL</sup>	1.8 <sup>F</sup>	7.8 <sup>L</sup>	134 <sup>D</sup>	7 <sup>K</sup>	18 <sup>C</sup>	122 bp
Baalbak (P <sub>5</sub> )	100 <sup>GH</sup>	28 <sup>CD</sup>	8.5 <sup>GH</sup>	17 <sup>BCD</sup>	31 <sup>JK</sup>	1.8 <sup>F</sup>	7.7 <sup>L</sup>	125 <sup>F</sup>	8 <sup>JK</sup>	16 <sup>DE</sup>	122 bp
Giza 164 (P <sub>6</sub> )	120 <sup>CD</sup>	19 <sup>FG</sup>	15.0 <sup>BC</sup>	22 <sup>A</sup>	56 <sup>CD</sup>	3.9 <sup>DE</sup>	29.4 <sup>F</sup>	75 <sup>L</sup>	9 <sup>J</sup>	9 <sup>GH</sup>	126 bp
Sakha 69 (P <sub>7</sub> )	80 <sup>I</sup>	29 <sup>BCD</sup>	8.5 <sup>GH</sup>	14 <sup>DE</sup>	51 <sup>DEF</sup>	5.3 <sup>ABC</sup>	34.8 <sup>C</sup>	88 <sup>J</sup>	10 <sup>HI</sup>	7 <sup>I</sup>	126 bp
Beni-suif (P <sub>8</sub> )	100 <sup>GH</sup>	19 <sup>EFG</sup>	9.3 <sup>FG</sup>	16 <sup>CD</sup>	54 <sup>CDE</sup>	5.0 <sup>BC</sup>	31.7 <sup>E</sup>	72 <sup>M</sup>	8 <sup>JK</sup>	9 <sup>GH</sup>	126 bp
Sohag 3 (P <sub>9</sub> )	98 <sup>H</sup>	20 <sup>EFG</sup>	9.7 <sup>FG</sup>	21 <sup>AB</sup>	59 <sup>C</sup>	4.9 <sup>BC</sup>	37.0 <sup>C</sup>	97 <sup>I</sup>	11 <sup>GH</sup>	10 <sup>G</sup>	126 bp
P <sub>6</sub> ×P <sub>1</sub>	119 <sup>CD</sup>	22 <sup>DEFG</sup>	11.0 <sup>EF</sup>	21 <sup>AB</sup>	17 <sup>M</sup>	3.7 <sup>DE</sup>	10.2 <sup>JK</sup>	85 <sup>K</sup>	9 <sup>J</sup>	17 <sup>CD</sup>	122 bp
P <sub>6</sub> ×P <sub>2</sub>	117 <sup>DE</sup>	25 <sup>CDEF</sup>	18.3 <sup>A</sup>	23 <sup>A</sup>	73 <sup>A</sup>	4.5 <sup>CD</sup>	48.0 <sup>A</sup>	97 <sup>I</sup>	9 <sup>J</sup>	8 <sup>HI</sup>	122 bp
P <sub>6</sub> ×P <sub>3</sub>	145 <sup>A</sup>	31 <sup>BC</sup>	16.0 <sup>B</sup>	21 <sup>AB</sup>	23 <sup>LM</sup>	3.4 <sup>E</sup>	11.3 <sup>J</sup>	141 <sup>C</sup>	16 <sup>D</sup>	25 <sup>A</sup>	122 bp
P <sub>1</sub> ×P <sub>6</sub>	110 <sup>EF</sup>	22 <sup>DEFG</sup>	10.0 <sup>FG</sup>	19 <sup>ABC</sup>	26 <sup>KL</sup>	3.9 <sup>DE</sup>	15.4 <sup>I</sup>	131 <sup>E</sup>	12 <sup>FG</sup>	16 <sup>DE</sup>	122 bp
P <sub>4</sub> ×P <sub>6</sub>	145 <sup>A</sup>	37 <sup>B</sup>	15.0 <sup>BC</sup>	22 <sup>A</sup>	48 <sup>FG</sup>	5.6 <sup>AB</sup>	43.9 <sup>B</sup>	131 <sup>E</sup>	25 <sup>B</sup>	21 <sup>B</sup>	122 bp
P <sub>7</sub> ×P <sub>1</sub>	105 <sup>FG</sup>	18 <sup>FG</sup>	13.3 <sup>CD</sup>	19 <sup>ABC</sup>	39 <sup>HI</sup>	5.5 <sup>AB</sup>	30.0 <sup>EF</sup>	144 <sup>B</sup>	21 <sup>C</sup>	15 <sup>E</sup>	122 bp
P <sub>7</sub> ×P <sub>2</sub>	85 <sup>I</sup>	55 <sup>A</sup>	11.0 <sup>EF</sup>	21 <sup>AB</sup>	44 <sup>GH</sup>	4.0 <sup>DE</sup>	35.0 <sup>CD</sup>	141 <sup>C</sup>	10 <sup>HI</sup>	10 <sup>G</sup>	126 bp
P <sub>7</sub> ×P <sub>3</sub>	105 <sup>FG</sup>	15 <sup>G</sup>	8.7 <sup>G</sup>	17 <sup>BCD</sup>	65 <sup>B</sup>	5.6 <sup>AB</sup>	27.0 <sup>G</sup>	121 <sup>G</sup>	13 <sup>EF</sup>	10 <sup>G</sup>	126 bp
P <sub>8</sub> ×P <sub>7</sub>	131 <sup>B</sup>	27 <sup>CDE</sup>	12.0 <sup>DE</sup>	21 <sup>AB</sup>	28 <sup>JKL</sup>	4.9 <sup>BC</sup>	18.3 <sup>H</sup>	47 <sup>N</sup>	9 <sup>J</sup>	18 <sup>C</sup>	126 bp
P <sub>8</sub> ×P <sub>1</sub>	125 <sup>BC</sup>	31 <sup>BC</sup>	15.3 <sup>B</sup>	22 <sup>A</sup>	49 <sup>EFG</sup>	4.0 <sup>DE</sup>	29.0 <sup>FG</sup>	119 <sup>G</sup>	21 <sup>C</sup>	13 <sup>F</sup>	122 bp
P <sub>9</sub> ×P <sub>1</sub>	140 <sup>A</sup>	28 <sup>CD</sup>	10.0 <sup>FG</sup>	23 <sup>A</sup>	49 <sup>EFG</sup>	5.9 <sup>A</sup>	37.0 <sup>C</sup>	134 <sup>D</sup>	17 <sup>D</sup>	7 <sup>I</sup>	122 bp
P <sub>4</sub> ×P <sub>6</sub>	115 <sup>DE</sup>	19 <sup>FG</sup>	15.0 <sup>BC</sup>	19 <sup>ABC</sup>	27 <sup>KL</sup>	4.6 <sup>CD</sup>	16.0 <sup>I</sup>	147 <sup>A</sup>	27 <sup>A</sup>	17 <sup>CD</sup>	122 bp
LSD	6.97	8.32	1.95	2.20	6.20	0.85	2.09	1.63	1.54	1.67	

In each column any two means having the same letter are not significantly different at the 5% level of significance, PH: Plant height, NT: No. of tillers, MSL: Main spike length, NS: No. of spikelets of the main spike, NG: No. of grains of main spike, GI: Grain index, PY: Plant yield, GPC: Grain protein content, GFeC: Grain iron content, GZnC: Grain zinc content

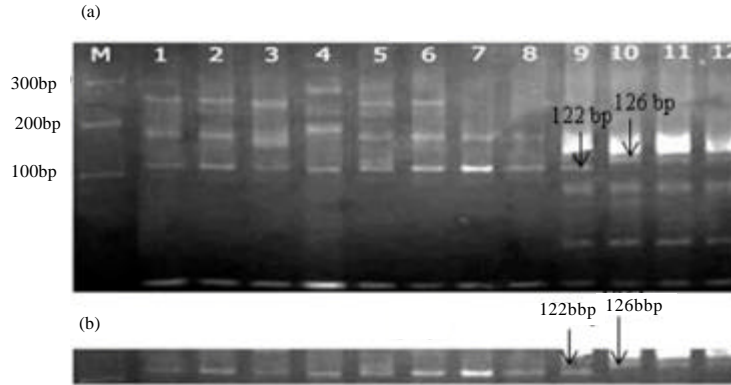


Fig. 1(a-b): (a) PCR analysis of Xuhw 89 marker in twelve wheat hybrids, (1)  $P_1 \times P_6$ , (2)  $P_6 \times P_1$ , (3)  $P_4 \times P_6$ , (4)  $P_6 \times P_2$ , (5)  $P_6 \times P_3$ , (6)  $P_7 \times P_1$ , (7)  $P_8 \times P_1$ , (8)  $P_9 \times P_1$ , (9)  $P_4 \times P_9$ , (10)  $P_7 \times P_2$ , (11)  $P_7 \times P_9$ , (12)  $P_5 \times P_7$ . M: 20 bp DNA ladder (Fermentas) and (b) Close up on the band of interest

grains per spike was higher in modern varieties than older ones. Contrary to grain yield and its components, protein content and majority of other quality parameters either did not change or decreased significantly during the same period.

#### Marker assisted selection for the Gpc-B1 gene in the hybrids using the Xuhw 89 marker:

In the present study 12 hybrids were obtained. The local cultivar Giza 164 ( $P_6$ ) was crossed with the four wild accessions Haifa ( $P_1$ ), Irbid ( $P_2$ ), Sweida ( $P_3$ ) and Rachaya ( $P_4$ ) and resulted in five hybrids. The cultivar Sakha 69 ( $P_7$ ) was crossed with the four wild accessions Haifa ( $P_1$ ), Irbid ( $P_2$ ), Sweida ( $P_3$ ) and Baalbak ( $P_8$ ) and produced four hybrids. While, one hybrid was obtained from crossing the Beni-suif 5 ( $P_9$ ) cultivar to the wild accession Haifa ( $P_1$ ) and two hybrids resulted from crossing Sohag 3 ( $P_9$ ) with the wild accessions Haifa ( $P_1$ ) and Rachaya ( $P_4$ ).

As shown in Fig. 1 PCR amplification of the  $F_1$  hybrid's genomic DNA using allele specific primers for the Xuhw 89 showed the presence of the 122 bp allele in all the hybrids except three, i.e., Sakha 69×Irbid ( $P_7 \times P_2$ ), Sakha 69×Sweida ( $P_7 \times P_3$ ) and Baalbak×Sakha 69 ( $P_8 \times P_7$ ). This allele has been previously assessed to be present in the dicoccoides wild genotypes while, the Egyptian hexaploid and tetraploid genotypes revealed the 126 bp allele (Hussein *et al.*, 2012).

#### Evaluation of the agronomic characters, grain protein content (GPC), grain iron content (GFeC) and grain zinc content (GZnC) in the hybrids and their performance:

Seven agronomic yield related traits in addition to the grain protein content (GPC), iron (GFeC) and zinc (GZnC) were evaluated in the  $F_1$  plants.

**Plant height (PH):** As shown in Table 3 significant differences in the mean plant height values were observed among the hybrids. The cross Sakha 69×Irbid ( $P_7 \times P_2$ ) revealed the lowest value of 85 cm followed by Sakha 69×Haifa ( $P_7 \times P_1$ ) and Sakha 69×Sweida ( $P_7 \times P_3$ ) which revealed a mean plant height value of 105 cm. While, the highest value for plant height was expressed by the crosses Giza 164×Sweida ( $P_6 \times P_3$ ) and Rachaya×Giza 164 ( $P_4 \times P_6$ ) which revealed a plant height of 145 cm. Concerning the hybrids obtained from crossing Giza 164 with the wild genotypes, the plant heights of  $F_1$  hybrids Giza 164×Haifa ( $P_6 \times P_1$ ) and Giza 164×Irbid ( $P_6 \times P_2$ ) were insignificantly

different from the parent Giza 164 but they were higher than the wild parent. The plant height of the hybrid Haifa×Giza 164 ( $P_1 \times P_6$ ) was equal to the  $P_1$  (Haifa) parent. While, the mean plant height in the two  $F_1$  hybrids Giza 164×Sweida ( $P_6 \times P_9$ ) and Rachaya×Giza 164 ( $P_4 \times P_6$ ) exceeded the height of the Giza 164 parent significantly indicating positive over-dominance.

Regarding the crosses between the local parent Sakha 69 ( $P_7$ ) and the wild genotypes, the mean plant height of the two hybrids Sakha 69×Haifa ( $P_7 \times P_1$ ) and Sakha 69×Irbid ( $P_7 \times P_2$ ) were in between the two parents, indicating partial or complete dominance as well as additive gene effects. While, the hybrids Sakha 69×Sweida ( $P_7 \times P_9$ ) and Baalbak×Sakha 69 ( $P_5 \times P_7$ ) were significantly taller than the higher parents ( $P_9$  and  $P_6$ ), thus revealing positive over-dominance. The three crosses including the local tetraploid genotypes Beni-suif 5 ( $P_8$ ) or Sohag 3 ( $P_9$ ) with the wild genotypes, i.e.,  $P_8 \times P_1$ ,  $P_9 \times P_1$  and  $P_4 \times P_9$  exhibited a higher mean value of plant height than the taller parents ( $P_1$  or  $P_4$ ) therefore, indicating a positive over-dominance.

**No. of tillers (NT):** Regarding No. of tillers, the crosses Sakha 69×Sweida ( $P_7 \times P_9$ ) and Sakha 69×Haifa ( $P_7 \times P_1$ ) showed the lowest values 15 and 18, respectively. Both crosses were significantly lower than their parents but they were insignificant from each other, thus revealing negative over-dominance. The highest mean value of No. of tillers was expressed by the cross Sakha 69×Irbid ( $P_7 \times P_2$ ) which revealed a mean value of 55. The mean value of the latter hybrid highly exceeded the higher parent Sakha 69 ( $P_7$ ) revealing positive over-dominance. The hybrid Baalbak×Sakha 69 ( $P_5 \times P_7$ ) displayed a mean No. of tillers of 27 which was insignificantly lower than the two parents exhibiting negative over-dominance. Concerning Giza 164 crosses with the wild genotypes, Rachaya×Giza 164 ( $P_4 \times P_6$ ) revealed the highest mean value of No. of tillers (37). No. of tillers of  $F_1$  hybrids Giza 164×Irbid ( $P_6 \times P_2$ ) and Giza 164×Sweida ( $P_6 \times P_9$ ) were 25 and 31, respectively and were insignificant from the wild parents Irbid ( $P_2$ ) and Sweida ( $P_9$ ). Finally, the mean No. of tillers of the cross Haifa×Giza 164 ( $P_1 \times P_6$ ) and its reciprocal Giza 164×Haifa ( $P_6 \times P_1$ ) was in between both parents, thus revealing partial or complete dominance as well as additive gene effects.

The crosses of the local cultivar Beni-suif 5 ( $P_8$ ) and Sohag 3 ( $P_9$ ) revealed positive over-dominance for Beni-suif 5×Haifa ( $P_8 \times P_1$ ), partial or complete dominance for the  $F_1$  cross Sohag 3×Haifa ( $P_9 \times P_1$ ). While, both mentioned crosses were insignificant from their wild parent Haifa ( $P_1$ ) as they gave 31 and 28, respectively but significantly higher than the local parents. Rachaya×Sohag 3 ( $P_4 \times P_9$ ) exhibited mean value of No. of tillers lower than both parents but it was insignificant from the lower parent Sohag 3 ( $P_9$ ) thus, showing negative over-dominance.

**Main spike length (MSL):** The highest mean value of main spike length (18.3 cm) was revealed by Giza 164×Irbid ( $P_6 \times P_2$ ) followed by Giza 164×Sweida ( $P_6 \times P_9$ ) (16 cm) as both showed positive over-dominance. Rachaya×Giza 164 ( $P_4 \times P_6$ ) exhibited main spike length mean value of 15 cm which was insignificant from the local cultivar. The two Giza 164  $F_1$  hybrids  $P_1 \times P_6$  and  $P_6 \times P_1$  revealed main spike length mean value insignificant from each other and in between the local Giza 164 ( $P_6$ ) and the wild parent Haifa ( $P_1$ ) therefore, indicating partial or complete over-dominance and additive gene effects. Concerning Sakha 69 ( $P_7$ ) hybrids, Sakha 69×Haifa ( $P_7 \times P_1$ ), Sakha 69×Irbid ( $P_7 \times P_2$ ) and Baalbak×Sakha 69 ( $P_5 \times P_7$ ) were significantly higher than their higher parent. The main spike length in these hybrids was 13.3, 11 and 12 cm, respectively, thus exhibiting positive over-dominance. Also, Sakha 69×Sweida ( $P_7 \times P_9$ ) was higher than the higher parent (Sakha 69) but the difference was not significant. Regarding the tetraploid cultivars Beni-suif 5 and Sohag 3 crosses, i.e.,  $P_8 \times P_1$ ,  $P_9 \times P_1$  and  $P_4 \times P_9$ , they exhibited a higher mean value of main spike length than the taller parents ( $P_1$  or  $P_4$ ), therefore, indicating a positive over-dominance (Table 3).



**No. of spikelets of main spike (NS):** Concerning mean values of No. of spikelets of the main spike as illustrated in Table 3, there were significant differences observed among the hybrids. The cross Sakha 69×Sweida ( $P_7 \times P_3$ ) revealed the lowest value of 17 revealing positive over-dominance. Also, the other hybrids of Sakha 69  $P_7 \times P_1$ ,  $P_7 \times P_2$  and  $P_5 \times P_7$  revealed positive over-dominance, where the mean value of the  $F_1$  hybrids was higher than the higher parent Sakha 69 (14), Irbid (15) and Baalbak (17), respectively. Regarding Giza 164  $F_1$  hybrids, the lowest mean value was 21 for Giza 164×Haifa ( $P_8 \times P_1$ ) and Giza 164×Sweida ( $P_8 \times P_3$ ). These two crosses with Haifa×Giza 164 ( $P_1 \times P_8$ ) exhibited mean values in between the wild and local parent, therefore, these crosses showed complete or partial dominance as well as additive gene effects. Only the  $F_1$  cross Giza 164×Irbid ( $P_8 \times P_2$ ) mean value was higher than the higher parent Giza 164 but it was not significant, thus it may be revealing positive over-dominance. The tetraploid crosses Beni-suif 5×Haifa ( $P_8 \times P_1$ ) and Sohag 3×Haifa ( $P_9 \times P_1$ ) mean values of No. of spikelets per main spike exceeded that of the higher parent indicating positive over-dominance. The  $F_1$  cross  $P_8 \times P_1$  mean value was highly significant than the higher parent Beni-suif 5. Rachaya×Sohag 3 ( $P_4 \times P_9$ ) and Rachaya×Giza 164 ( $P_4 \times P_8$ ) exhibited mean value typical to the wild parent (19) and local parent (22), respectively.

**No. of grains of main spike (NG):** Data presented in Table 3 illustrated that among Giza 164 ( $P_8$ ) crosses, Giza 164×Haifa ( $P_8 \times P_1$ ) resulted in the lowest No. of grains of main spike (17) followed by Giza 164×Sweida ( $P_8 \times P_3$ ) of 23, thus revealing mean values significantly lower than the lower parent indicating negative over-dominance. Giza 164×Irbid ( $P_8 \times P_2$ ) was the highest cross mean value of (73), exceeding the higher parent Giza 164 significantly, indicating positive over-dominance. Haifa×Giza 164 ( $P_1 \times P_8$ ) and Rachaya×Giza 164 ( $P_4 \times P_8$ ) revealed values in between the wild and the local parent, therefore, indicating partial or complete dominance and additive gene effects. Concerning the  $F_1$  cross Haifa×Giza 164 ( $P_1 \times P_8$ ) mean value was insignificantly higher than the wild parent Haifa ( $P_1$ ). Also, among Sakha 69  $F_1$  crosses, Sakha 69×Haifa ( $P_7 \times P_1$ ) and Sakha 69×Irbid ( $P_7 \times P_2$ ) exhibited values in between their parents therefore; they reveal partial or complete dominance. While, Sakha 69×Sweida ( $P_7 \times P_3$ ) the No. of grains per main spike value of 65 was significantly higher than the local and the wild parent, thus indicating positive over-dominance. Baalbak×Sakha 69 ( $P_5 \times P_7$ ) was insignificantly lower than the lower parent Baalbak ( $P_5$ ). Similarly, the tetraploid cross Rachaya×Sohag 3 ( $P_4 \times P_9$ ), thus revealing negative over-dominance. The other two tetraploid  $F_1$  crosses Beni-suif 5×Haifa ( $P_8 \times P_1$ ) and Sohag 3×Haifa ( $P_9 \times P_1$ ) exhibited mean values in between their local and wild parents indicating partial or complete over-dominance as well as additive gene effects.

**Grain index (100 grain weight):** Regarding the weight of 100 grains of Giza 164, the  $F_1$  hybrid Rachaya×Giza 164 ( $P_4 \times P_8$ ) was the highest among the hybrids of (5.6 g) followed by Giza 164×Irbid ( $P_8 \times P_2$ ) of (4.5 g). They were higher than both parents although the first cross significantly exceeded the higher parent; the two  $F_1$  crosses revealed positive over-dominance. The other three hybrids Giza 164×Sweida ( $P_8 \times P_3$ ), Giza 164×Haifa ( $P_8 \times P_1$ ) and Haifa×Giza 164 ( $P_1 \times P_8$ ) were similar to the local cultivar Giza 164 ( $P_8$ ) of (3.4), (3.7) and (3.9) respectively, i.e., significantly higher than the lower wild parent. Therefore, these  $F_1$  crosses revealed partial or complete over-dominance and additive gene effects. Concerning Sakha 69 hybrids, all the resultant hybrids mean values were significantly exceeding their wild parent. Sakha 69×Haifa ( $P_7 \times P_1$ ) and Sakha×Sweida ( $P_7 \times P_3$ ) were insignificant from the local cultivar Sakha 69 ( $P_7$ ).

They displayed grain index of 5.5 and 5.6 g, respectively, thus revealing positive over-dominance. Sakha 69×Irbid ( $P_7 \times P_2$ ) and Baalbak×Sakha 69 ( $P_5 \times P_7$ ) exhibited mean values in between their wild and local parents of 4 and 4.9 g, respectively, indicating partial or complete dominance as well as additive gene effects. The three crosses including the local tetraploid genotypes Beni-suif 5 and Sohag 3  $P_8 \times P_1$  and  $P_4 \times P_9$  significantly exceeded the wild genotype, their lower value parent, thus revealing partial or complete dominance and additive gene effects. While,  $P_9 \times P_1$  was significantly higher than Sohag 3 ( $P_9$ ) and Haifa ( $P_1$ ) revealing positive over-dominance and also revealing the highest value among the hybrids (5.9 g).

**Plant yield (Total grain weight in g plant<sup>-1</sup>):** As shown in Table 3 among the Giza 164 hybrids, Giza 164×Irbid ( $P_6 \times P_2$ ) and Rachaya ( $P_4 \times P_6$ ) were the highest resulting in plant yield of 48 and 43.9 g, respectively. They significantly exceeded the highest parent, thus revealing positive over-dominance. 164 ( $P_1 \times P_6$ ) were in between their local and wild parents expressing 10.2, 11.3 and 15.4 grams, respectively, indicating partial or complete dominance as well as additive gene effects.

Concerning the local cultivar Sakha 69  $F_1$  crosses, Sakha 69×Irbid ( $P_7 \times P_2$ ) revealed the highest mean value of plant yield of 35 g. It was significantly higher than the wild parent and insignificantly higher than the local parent Sakha 69. Sakha 69×Irbid ( $P_7 \times P_2$ ), Sakha 69×Sweida ( $P_7 \times P_3$ ) and Baalbak×Sakha 69 ( $P_5 \times P_7$ ) were significantly higher from their wild parents but lower than the local cultivar Sakha 69 ( $P_7$ ) producing 30, 26.9 and 18.3 g, respectively, thus exhibiting partial or complete dominance as well as additive gene effects. Regarding the three crosses obtained from crossing the local tetraploid genotypes Beni-suif 5 ( $P_8$ ) or Sohag 3 ( $P_9$ ) with the wild genotypes, i.e., Beni-suif 5×Haifa ( $P_8 \times P_1$ ) and Rachaya×Sohag 3 ( $P_4 \times P_9$ ) revealed plant yield mean value in between parents indicating partial or complete dominance and additive gene effects. While, Sohag 3×Haifa ( $P_9 \times P_1$ ) exhibited mean value similar to the local parent genotype.

**Grain protein content (GPC, g kg<sup>-1</sup>):** The grain protein content of Giza 164 crosses ranged from 85-41 gm kg<sup>-1</sup>. Giza 164×Haifa ( $P_6 \times P_1$ ), Giza 164×Irbid ( $P_6 \times P_2$ ) and Rachaya×Giza 164 ( $P_4 \times P_6$ ) revealed mean values in between the two parents, indicating partial or complete dominance as well as additive gene effects. While, Haifa×Giza 164 ( $P_1 \times P_6$ ) exhibited mean value significantly exceeding the higher parent ( $P_1$ ), thus revealing positive over-dominance. The grain protein content of the hybrid Giza 164×Sweida ( $P_6 \times P_3$ ) was equal to the Sweida ( $P_3$ ) parent. The lowest value of grain protein content of crosses obtained from Sakha 69 and the wild genotypes was 47 gm kg<sup>-1</sup> for Baalbak×Sakha 69 ( $P_5 \times P_7$ ). This value was lower than the two parents revealing negative over-dominance and it was the only cross showing negative over-dominance. On the contrary, Sakha 69×Haifa ( $P_7 \times P_1$ ) and Sakha 69×Irbid ( $P_7 \times P_2$ ) exhibited values higher than the high parent Haifa and Irbid ( $P_1$  and  $P_2$ ), indicating positive over-dominance. Sakha 69×Sweida ( $P_7 \times P_3$ ) exhibited mean value of grain protein content in between the two parents of 121 gm kg<sup>-1</sup> therefore, revealing partial or complete dominance as well as additive gene effects. The three crosses including the local tetraploid genotypes Beni-suif 5 ( $P_8$ ) or Sohag 3 ( $P_9$ ) with the wild genotypes, i.e.,  $P_8 \times P_1$ ,  $P_9 \times P_1$  and  $P_4 \times P_9$  exhibited higher mean value of grain protein content than the highest parent ( $P_1$  and  $P_4$ ) therefore, indicating positive over-dominance.

**Grain iron content (GFeC, mg kg<sup>-1</sup>):** The three Giza 164 ( $P_6$ ) crosses with the wild genotypes, i.e., Giza 164×Haifa ( $P_6 \times P_1$ ), Giza 164×Irbid ( $P_6 \times P_2$ ) and Giza 164×Sweida ( $P_6 \times P_3$ ) revealed mean values equal to one of the parents. The first two crosses were equal to Giza 164 ( $P_6$ ) and the third was equal to the wild parent Sweida ( $P_3$ ). Haifa×Giza 164 ( $P_1 \times P_6$ ) showed mean value insignificant

from the wild genotype but highly significant from the local parent cultivar, indicating partial or complete over-dominance and additive gene effects. Rachaya×Giza 164 ( $P_4 \times P_6$ ) exhibited grain iron content mean value significantly higher than the two parents therefore, revealing positive over-dominance. Concerning the crosses obtained from crossing the local cultivar Sakha 69 with the wild genotypes, Sakha 69×Haifa ( $P_7 \times P_1$ ) exhibited the highest value exceeding the two parents and indicating positive over-dominance. Sakha 69×Sweida ( $P_7 \times P_3$ ) and Baalbak×Sakha 69 ( $P_5 \times P_7$ ) showed values inbetween the two parents, thus revealing partial or complete over-dominance and additive gene effects. Sakha 69×Irbid ( $P_7 \times P_2$ ) exhibited mean value of grain iron content insignificant from the local cultivar parent ( $P_7$ ). The three crosses obtained from crossing the tetraploid cultivar Beni-suif 5 ( $P_8$ ) or Sohag 3 ( $P_9$ ) with the wild genotypes exhibited mean value exceeding the high parents ( $P_1$  and  $P_9$ ) showing positive over-dominance.

**Grain zinc content (GZnC, mg kg<sup>-1</sup>):** Concerning crosses obtained from crossing the local parent Giza 164 with the wild genotypes. The crosses  $P_6 \times P_1$ ,  $P_6 \times P_3$ ,  $P_1 \times P_6$  and  $P_4 \times P_6$  exhibited values exceeding the parents with the highest mean value, indicating positive over-dominance. Giza 164×Irbid is the only cross exhibiting mean of grain zinc content lower than the two parents although it was insignificant from the low valued local parent, thus revealing negative over-dominance. Similarly, Baalbak×Sakha 69 ( $P_5 \times P_7$ ) revealed mean value higher than the two parents of 18 mg kg<sup>-1</sup> therefore, showing positive over-dominance. While, the crosses Sakha 69×Irbid ( $P_7 \times P_2$ ) and Sakha 69×Sweida ( $P_7 \times P_3$ ) exhibited equal mean values of 10 mg kg<sup>-1</sup> in between the two parents, indicating partial or complete over-dominance as well as additive gene effects. Sakha 69×Haifa ( $P_7 \times P_1$ ) showed grain zinc content mean value equal to the wild parent Haifa ( $P_1$ ). Regarding the tetraploid crosses, Beni-suif 5×Haifa ( $P_8 \times P_1$ ) and Rachaya×Sohag 3 ( $P_4 \times P_9$ ) exhibited mean values in between the two parents, indicating partial or complete dominance and additive gene effects. Sohag 3×Haifa ( $P_9 \times P_1$ ) is the only cross among the tetraploid crosses that exhibited mean value lower than the two parents, thus revealing negative over-dominance.

Analysis of variance for the studied seven agronomic traits in addition to the grain protein, iron and zinc content indicated significant mean squares due to genotypes for No. of tillers (Table 4). While, highly significant mean squares existed for all the other traits. Non-significant differences were observed among replicates for the studied traits. These results point out the effect of the genotypes on these traits.

In comparison to the present results, Cakmak *et al.* (2004) reported that the differences in Fe concentration among the DIC chromosome lines were less in comparison to those found for Zn concentration. Distelfeld *et al.* (2006a) used recombinant chromosome substitution lines (RSLs) and identified the effects of the Gpc-B1 locus on grain micronutrient concentrations. Where, carrying the Gpc-B1 allele of *T. dicoccoides* accumulated on average 12% higher concentration of Zn, 18% higher concentration of Fe, 29% higher concentration of Mn and 38% higher concentration of protein in the grain as compared with RSLs carrying the allele from cultivated wheat (*Triticum durum*). Also, (Brevis and Dubcovsky, 2008) described the effect of the introgression of the DIC chromosome 6BS segment including Gpc-B1 on grain yield, thousand grain weight (TGW) and protein yield (grain yield by grain protein content) in hexaploid and tetraploid wheat. Durum lines carrying the DIC Gpc-B1 allele showed average grain yield reductions between 6 and 17% and 8% reduction in TGW. Hexaploid lines carrying the DIC Gpc-B1 allele showed non-significant differences in grain yield compared to the corresponding recurrent parents. The presence of significant gene by variety interactions indicated that the effect of the Gpc-B1 region varies across genotypes.

Table 4: Analysis of variance for seven agronomic characters and grain protein, iron and zinc content in the nine parental genotypes and their hybrids

S.O.V.	d.f	Mean squares					
		PH	NT	MSL	NS	NG	F 0.05
Genotypes	20	947.3**	217.7*	40.3**	28.6**	741.4**	1.84
Replicates	2	56.7 <sup>ns</sup>	12.5 <sup>ns</sup>	0.25 <sup>ns</sup>	6.7 <sup>ns</sup>	0.82 <sup>ns</sup>	3.23
Error	40	18.3	25.4	1.4	1.9	14.1	
Total	62						
S.O.V.	d.f	GI	PY	GPC	GFeC	GZnC	F 0.05
Genotypes	20	5.3**	522.6**	2368.3**	104.9**	67.7**	1.84
Replicates	2	0.15 <sup>ns</sup>	0.42 <sup>ns</sup>	0.047 <sup>ns</sup>	2.33 <sup>ns</sup>	0.19 <sup>ns</sup>	3.23
Error	40	0.26	1.6	1.04	0.93	1.04	
Total	62						

PH: Plant height, NT: No. of tillers, MSL: Main spike length, NS: No. of spikelets of the main spike, NG: No. of grains of main spike, GI: Grain index, PY: Plant yield, GPC: Grain protein content, GFeC: Grain iron content, GZnC: Grain zinc content

### HETEROTIC EFFECTS

**Mid and high parent heterosis percent of the seven agronomic characters, grain protein content (GPC), grain iron content (GfeC) and grain zinc content (GznC):** In crop improvement programs, the phenomenon of heterosis has been extensively exploited in different crop plants and led to significant increase in yield production.

**Mid-parents heterosis (MPH):** As shown in Table 5 highly significant percentages of heterosis relative to mid-parent were exhibited for plant height in eight crosses ( $P_6 \times P_3$ ,  $P_4 \times P_6$ ,  $P_7 \times P_1$ ,  $P_7 \times P_3$ ,  $P_5 \times P_7$ ,  $P_8 \times P_1$ ,  $P_9 \times P_1$  and  $P_4 \times P_9$ ). For No. of tillers, five crosses ( $P_6 \times P_2$ ,  $P_6 \times P_3$ ,  $P_4 \times P_6$ ,  $P_7 \times P_2$ ,  $P_8 \times P_1$  and  $P_9 \times P_1$ ) revealed positive highly significant heterosis relative to mid-parent and all crosses revealed highly significant positive mid-parent heterosis for main spike length and No. of spikelets except ( $P_1 \times P_6$ ) and ( $P_4 \times P_9$ ), respectively. For the No. of grains all crosses revealed highly significant positive heterosis except ( $P_6 \times P_3$ ,  $P_1 \times P_6$ ,  $P_5 \times P_7$  and  $P_4 \times P_9$ ). Also, grain index showed positive highly significant heterosis for all the crosses. It is worthy to note that the crosses  $P_4 \times P_6$ ,  $P_7 \times P_1$  and  $P_8 \times P_1$  showed positive highly significant mid-parent heterosis for grain yield per plant grain protein, iron and zinc content.

The cross  $P_9 \times P_1$  revealed highly significant positive heterosis for plant yield, grain protein and iron content but exhibited negative highly significant heterosis for grain zinc content. For the crosses  $P_7 \times P_2$  and  $P_7 \times P_3$  plant yield and grain protein content traits exhibited positive highly significant heterosis. While grain iron and zinc content revealed negative value of heterosis. Moreover, the cross  $P_6 \times P_2$  showed positive highly significant heterosis only for grain yield per plant and negative highly significant heterosis for the other three traits. On the contrary, the crosses  $P_6 \times P_3$ ,  $P_1 \times P_6$  and  $P_4 \times P_9$  revealed positive highly significant heterosis for grain protein, iron and zinc content but undesirable negative highly significant heterosis for plant yield. Finally, the two crosses  $P_6 \times P_1$  and  $P_5 \times P_7$  showed undesirable highly significant negative heterosis for the three traits plant yield, grain protein and iron content. Grain zinc content displayed highly significant positive heterosis in these two crosses.

Table 5: Heterosis (%) relative to mid parent (MPH) and high parent (HPH) for studied traits of the twelve wheat crosses

Crosses	PH		NT		MSL		NS		NG	
	M.P.H.	H.P.H.	M.P.H.	H.P.H.	M.P.H.	H.P.H.	M.P.H.	H.P.H.	M.P.H.	H.P.H.
<b>Giza 164 (P<sub>6</sub>) hybrids</b>										
P <sub>6</sub> ×P <sub>1</sub>	3.48	8.18*	-10.20*	-26.60**	2.32*	-26.6**	27.27**	-4.54**	56.95**	-69.64**
P <sub>6</sub> ×P <sub>2</sub>	6.10	16.70**	11.10**	-3.85	68.66**	22.00**	24.34**	4.54**	78.04**	30.35**
P <sub>6</sub> ×P <sub>3</sub>	24.13**	45.00**	24.00**	0.00	54.58**	6.67**	20.00**	-4.54**	-48.89**	-58.39**
P <sub>1</sub> ×P <sub>6</sub>	-4.35	0.00	-10.20*	-26.70**	-6.97**	-33.33**	15.15**	-13.63**	-34.17**	-53.57**
P <sub>4</sub> ×P <sub>6</sub>	31.80**	45.00**	68.18**	48.00**	42.85**	0.00	7.31**	0.00	14.28**	-14.28**
<b>Sakha 69 (P<sub>7</sub>) hybrids</b>										
P <sub>7</sub> ×P <sub>1</sub>	10.53**	31.25**	-38.98**	-40.00**	77.3**	56.47**	52.71**	35.71**	5.40	-23.52**
P <sub>7</sub> ×P <sub>2</sub>	-5.55	6.25	100.00**	89.66**	44.74**	29.41**	44.82**	40.00**	14.35**	-13.72**
P <sub>7</sub> ×P <sub>3</sub>	16.67**	31.25**	-50.00**	-51.64**	22.5**	2.35*	25.92**	21.42**	52.94**	27.45**
P <sub>5</sub> ×P <sub>7</sub>	45.56**	63.75**	-5.26	-6.84	41.18**	41.18**	35.48**	23.52**	-31.70**	-45.10**
<b>T. durum hybrids</b>										
P <sub>3</sub> ×P <sub>1</sub>	19.04**	25.00**	26.44**	3.33	93.67**	64.52**	62.96**	37.50**	27.27**	-9.26**
P <sub>3</sub> ×P <sub>1</sub>	34.62**	42.86**	12.00**	-6.67	23.46**	3.10**	43.75**	9.52**	19.51**	-16.94**
P <sub>4</sub> ×P <sub>9</sub>	16.16**	17.34**	-13.60**	-20.08**	91.08**	54.64**	-5.00**	-9.52**	-37.93**	-54.23**
LSD 0.05	6.97		8.32		1.95		2.20		6.20	
LSD 0.01	9.00		10.61		2.49		2.90		7.90	
Crosses	GI		PY		GPC		GFeC		GZnC	
<b>Giza 164 (P<sub>6</sub>) hybrids</b>										
P <sub>6</sub> ×P <sub>1</sub>	17.46**	-5.13**	-47.72**	-65.30**	-7.60**	-22.02**	-18.18**	-30.77**	41.67**	13.33**
P <sub>6</sub> ×P <sub>2</sub>	40.62**	15.38**	154.64**	63.27**	-5.82**	-25.95**	-21.7**	-35.71**	-36.00**	-50.00**
P <sub>6</sub> ×P <sub>3</sub>	17.24**	-12.82**	-39.41**	-61.56**	30.55**	0.00	28.00**	0.00	108.3**	66.67**
P <sub>1</sub> ×P <sub>6</sub>	23.80**	0.00	-20.62**	-47.62**	42.39**	20.18**	9.09**	-7.69**	33.33**	6.67**
P <sub>4</sub> ×P <sub>6</sub>	96.49**	43.59**	32.70**	49.32**	25.36**	-2.24**	212.5**	177.77**	55.55**	16.67**
<b>Sakha 69 (P<sub>7</sub>) hybrids</b>										
P <sub>7</sub> ×P <sub>1</sub>	42.85**	3.77**	35.75**	-13.79**	45.88**	32.11**	82.61**	61.54**	36.36**	0.00
P <sub>7</sub> ×P <sub>2</sub>	2.56**	-24.53**	62.41**	0.57	28.76**	7.63**	-16.67**	-28.57**	-13.04**	-37.5**
P <sub>7</sub> ×P <sub>3</sub>	55.56**	5.66**	26.46**	-22.41**	5.68**	-14.18**	0.00	-18.75**	-9.09**	-33.33**
P <sub>5</sub> ×P <sub>7</sub>	38.02**	-7.55**	-13.88**	-47.41**	-55.87**	-62.4**	0.00	-10.00**	56.52**	12.50**
<b>T. durum hybrids</b>										
P <sub>3</sub> ×P <sub>1</sub>	8.11**	-20.00**	41.11**	-8.83**	31.49**	9.17**	100**	61.54**	8.33**	-13.33**
P <sub>3</sub> ×P <sub>1</sub>	61.64**	20.41**	59.48**	0.00	30.10**	22.94**	41.67**	30.77**	-44.00**	-53.33**
P <sub>4</sub> ×P <sub>9</sub>	37.31**	-7.54**	-28.57**	-56.75**	27.27**	9.70**	200.00**	145.45**	21.43**	-5.55**
LSD 0.05	0.85		2.09		1.63		1.54		1.67	
LSD 0.01	1.07		2.66		2.15		2.03		2.15	

(\*) and (\*\*) Correlation is significant at the 0.05 level and at the 0.01 level respectively, PH: Plant height, NT: No. of tillers, MSL: Main spike length, NS: No. of spikelets of the main spike, NG: No. of grains of main spike, GI: Grain index, PY: Plant yield, GPC: Grain protein content, GFeC: Grain iron content, GZnC: Grain zinc content

**High-parent heterosis (HPH):** The significant superiority of the F<sub>1</sub> hybrids over the better or high parent performance (heterobeltosis) is the critical test for the F<sub>1</sub> crosses. The heterotic effects of different character relative to the better parent (HPH) are presented in Table 5. It could be noticed that significant and highly significant parent heterosis were observed for plant height in all the crosses except P<sub>1</sub>×P<sub>6</sub> and P<sub>7</sub>×P<sub>2</sub>. This is undesirable as it shows that the crosses revealed plant height higher than that of the better parent. Two crosses (P<sub>4</sub>×P<sub>6</sub> and P<sub>7</sub>×P<sub>2</sub>) revealed positive highly

significant heterosis relative to the better parent for No. of tillers. All crosses except three ( $P_1 \times P_6$ ,  $P_6 \times P_1$  and  $P_4 \times P_9$ ) exhibited significant and highly significant desirable high parent heterosis for main spike length. For No. of spikelets in the main spike, seven crosses ( $P_6 \times P_2$ ,  $P_7 \times P_1$ ,  $P_7 \times P_2$ ,  $P_7 \times P_3$ ,  $P_5 \times P_7$ ,  $P_8 \times P_1$  and  $P_9 \times P_1$ ) expressed desirable highly significant high parent heterosis. While, only two crosses ( $P_6 \times P_2$  and  $P_7 \times P_3$ ) revealed desirable highly significant high parent heterosis for No. of grains per main spike. For grain index five crosses ( $P_6 \times P_2$ ,  $P_4 \times P_6$ ,  $P_7 \times P_1$ ,  $P_7 \times P_3$  and  $P_9 \times P_1$ ) expressed highly significant high parent heterosis. Regarding grain yield per plant (PY), only the two crosses ( $P_6 \times P_2$  and  $P_4 \times P_6$ ) showed desirable highly significant high parent heterosis and the two crosses ( $P_7 \times P_2$  and  $P_9 \times P_1$ ) revealed insignificant high parent heterosis.

Concerning the grain protein content six crosses revealed positive highly significant heterosis, i.e.,  $P_1 \times P_6$ ,  $P_7 \times P_1$ ,  $P_7 \times P_2$  and the *T. durum* hybrids ( $P_8 \times P_1$ ,  $P_9 \times P_1$  and  $P_4 \times P_9$ ). Also, for grain iron content  $P_4 \times P_6$ ,  $P_7 \times P_1$  and the *T. durum* crosses ( $P_8 \times P_1$ ,  $P_9 \times P_1$  and  $P_4 \times P_9$ ) exhibited highly significant high parent heterosis. While, the other crosses revealed highly significant negative heterosis or zero values, i.e.,  $P_6 \times P_8$  expressed grain iron content value equal to the high parent. On the contrary, *T. durum* hybrids expressed negative highly significant heterosis for grain zinc content. Also,  $P_6 \times P_2$ ,  $P_7 \times P_2$  and  $P_7 \times P_3$  showed negative highly significant heterosis for this trait. While the other crosses  $P_6 \times P_1$ ,  $P_6 \times P_3$ ,  $P_1 \times P_6$ ,  $P_4 \times P_6$  and  $P_5 \times P_7$  expressed positive highly significant values of heterosis.

**Correlation coefficients between plant yield (PY), grain protein content (GPC), grain iron content (GF<sub>Fe</sub>C) and grain zinc content (GznC):** As illustrated in Table 6 correlation coefficient between Giza 164 ( $P_6$ ) and its crosses, Sakha 69 ( $P_7$ ) and its crosses, *T. durum* parents and their resultant crosses and the nine parental wheat genotypes and the twelve crosses was calculated. In general, there was negative correlation between plant yield, grain protein, iron and zinc content. Although it was significant only between plant yield and grain protein content for Giza 164 ( $P_6$ ) and its crosses. Similarly, Blanco *et al.* (2002) found that grain protein content was negatively correlated with grain yield. They attributed the negative yield - protein correlation to environmental factors, genetic components, dilution of grain nitrogen with a much larger grain biomass accumulation or to bioenergetic requirements for synthesis of carbohydrates and proteins. They added that in some cases different loci within the linkage groups could be involved. Also, the results of Gonzalez-Hernandez *et al.* (2004) and Blanco *et al.* (2012) revealed that grain protein concentration showed negative correlation with grain yield in all environments. Moreover, Oda *et al.* (2008) found that the grain protein content was not correlated with the thousand kernel weight. Therefore, the high grain protein content did not result from low thousand kernel weight. They added that the Gpc-B1 gene increased not only the protein content but also the zinc and iron content. In addition, Suprayogi *et al.* (2009) detected significant negative correlation between GPC and kernel weight, indicating the presence of genetic factors that are likely pleiotropic on GPC. They pointed out despite these negative correlations, only three GPC QTL were associated with kernel weight. In contrast, the results of Groos *et al.* (2003) revealed no negative relationships between QTLs for yield and GPC. Also there was highly significant negative correlation between plant yield and grain zinc content for all genotypes except for Giza 164 ( $P_6$ ) where it was negative but insignificant. In this context, Tiwari *et al.* (2009) found non-significant correlation between 100 grain weight and the grain iron (Fe) and zinc (Zn) concentrations with r varying between 0.0 and 0.15.

Table 6: Correlation coefficients between plant yield, GPC, GFeC and GZnC in *T. aestivum* and *T. durum* cultivars and their hybrids

Genotypes/characters	Giza 164 (P <sub>6</sub> )			
	PY	GPC	GFeC	GZnC
PY	1.000	-0.593*	-0.173	-0.505
GPC	-	1.000	0.614*	0.758**
GFeC	-	-	1.000	0.490
GZnC	-	-	-	1.000
<b>Sakha 69 (P<sub>7</sub>)</b>				
PY	1.000	-0.269	-0.185	-0.784**
GPC	-	1.000	0.633*	0.314
GFeC	-	-	1.000	0.065
GZnC	-	-	-	1.000
<b><i>T. durum</i> (P<sub>8</sub> and P<sub>9</sub>)</b>				
PY	1.000	-0.397	0.060	-0.867**
GPC	-	1.000	0.562	0.511
GFeC	-	-	1.000	0.051
GZnC	-	-	-	1.000
<b>Parental genotypes and their hybrids</b>				
PY	1.000	-0.213	0.141	-0.557**
GPC	-	1.000	0.596**	0.316*
GFeC	-	-	1.000	0.148
GZnC	-	-	-	1.000

(\*) And (\*\*) correlation is significant at the 0.05 level and at the 0.01 level respectively

Concerning correlation between grain protein content and grain iron content it was significant only for Giza 164 (P<sub>6</sub>) and its crosses and Sakha 69 (P<sub>7</sub>) and its crosses and insignificant for *T. durum* parents and their crosses. In general, for all the genotypes there was highly significant correlation between the two traits. Regarding the grain protein content and grain zinc content highly significant correlation was revealed for Giza 164 (P<sub>6</sub>) and its crosses but insignificant for the other two genotypes Sakha 69 (P<sub>7</sub>) and *T. durum* and their crosses. Thus, according to data revealed from the nine wheat parental genotypes and their twelve crosses the correlation between these two traits was significant. In this context, Peleg *et al.* (2009) detected significant positive correlation between Grain Protein Concentration (GPC), Zn, Fe and Cu which was supported by significant overlap between the respective QTLs, suggesting common physiological and/or genetic factors controlling the concentrations of these mineral nutrients.

Correlation between grain iron and zinc content was weak positive and insignificant. This is in good agreement with Tiwari *et al.* (2009). They stated that no significant correlation was observed between iron (Fe) and zinc (Zn) concentration in the grains, indicating that grain iron and zinc accumulation may be controlled by different loci.

The present results confirmed that the Grain Protein Content (GPC) is a quantitative trait that is affected by major and minor genes. Moreover, the results suggest that the minor genes affecting grain iron and zinc content are not transferred in all the hybrids together with the major genes that affect the grain protein content and that a weak negative correlation exists between grain yield and grain protein content. Nevertheless, there is a great potential to improve the Grain Protein Content (GPC) of certain wheat genotypes without affecting the grain yield. As some hybrids proved to be promising such as: P<sub>4</sub>×P<sub>6</sub> which exhibited an increase in grain yield, grain

protein, iron and zinc contents. Also, the hybrid  $P_8 \times P_2$  revealed an increase in grain yield and grain protein content while, the iron and zinc contents were not affected. Moreover, in the hybrid  $P_9 \times P_1$ , there was an increase in the grain protein and iron contents without affecting the grain yield.

In conclusion, the results of the present investigation confirmed the usefulness of the molecular markers and marker assisted selection in the wheat breeding programs. This approach permitted to assess the genetic variability among the studied genotypes, to assess the presence of the high Gpc-B1 allele in the wild accessions and to detect the introgression of this wild-type allele into Egyptian durum and bread wheat cultivars. Therefore, these results could be considered as a first step in improving the nutritional quality of Egyptian wheat cultivars via biofortification.

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