# Combining Ability and Heterosis Relative to RAPD Marker in Cultivated and Newly Hexaploid Wheat Varieties

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**Abstract:** Combining ability and heterosis were studied in a 7x7 diallel set of bread wheat. Analysis of variance revealed that mean squares due to genotypes, parents and $F_1$ crosses, were highly significant for all studied characters. The mean squares due to both general (GCA) and specific (SCA) combining abilities were highly significant for all studied characters. Significant and highly significant desirable heterosis relative to the better parent was recorded in all studied characters. The maximum useful heterosis (78.05%) was observed in grain yield/plant for the cross ($P_4xP_7$). Many hybrids showed desirable significant or highly significant specific combining ability effects for all studied traits, except days to 50% heading and plant height. The genetic relationships among the seven parental genotypes were investigated using 10 RAPD primers. The highest similarity index was found between $P_5$ and $P_6$, while the lowest similarity index was found between $P_2$ and $P_6$. Among the studied parents, five out of seven, were characterized by 13 negative and 2 positive unique markers. Non significant correlations were observed between genetic distance with both of the amount of heterosis and SCA as well as between heterosis with SCA for grain yield/plant.

**Key words:** Bread wheat, hybrids, combining ability, heterosis, DNA, unique marker, RAPD, similarity index.

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important and strategic crops all over the world. In Egypt, wheat is the main cereal crop used as a stable food for urban and rural societies and the major source of straw for animal feeding. However, total wheat consumption has drastically increased due to over population growth by about 2.5% per year. Egypt imports about 45% of its wheat requirements. This reflects the size of the problem and the efforts needed to increase wheat production (Gad, 2010). Thus, increasing production per unit area appears to be one of the important factors for narrowing the gap between wheat production and consumption.

During breeding programs, it is necessary to select pure lines of high general combining ability (GCA) that indicates the additive gene effect. On the base of that, predicting progenies and making choice of cross combinations and genotypes can be carried out. Combining ability investigations carried out by breeders to select parents with efficient transferring desirable genes to the progenies (Madic *et al*., 2005). Singh, 2012 reported that, general and specific combining ability effects are very important in constructing the next phase of a breeding programme.

Genetic diversity is one of the key factors for the improvement of many crop plants including wheat. Different molecular markers techniques such as, restriction fragment length polymorphism (RFLP; Paull *et al*. 1998), simple sequence repeat (SSR; Plaschke *et al*. 1995), sequence tagged site (STS; Talbert *et al*. 1994) and amplified fragment length polymorphism (AFLP; Barret and Kidwell, 1998) have been used to estimate the genetic diversity in wheat. The random amplified polymorphic DNA (RAPD) technique, regardless of its sensitivity to reaction conditions and problems with repeatability and amplifying of non-homologous sequences (Devos and Gale, 1992). The RAPD technique has been successfully used for the assessment of genetic diversity in hexaploid wheat (Liu *et al*., 1999; Sivolap *et al*., 1999; Maric *et al*., 2004; Abd-El-Haleem *et al*., 2009; Nimbal *et al*. 2009 and Saleh, 2012).

Evaluation of hybrids for heterosis or combining ability in the field is both expensive and time consuming. For this reason, other parameters such as pedigree information, qualitative and quantitative traits and biochemical data were adapted to study heterosis (Wang *et al.*, 1992). RAPD marker has also been extensively used to correlate genetic diversity and heterosis in wheat (Liu *et al*. 1999, Bruel *et al.* 2006 and El-Maghraby *et al.* 2010).

The aims of this research were: 1) Estimation of heterosis and type of gene action in diallel crosses among seven hexaploid wheat varieties and lines, 2) Studying the genetic diversity among the studied wheat genotypes

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via RAPD analysis and 3) Examining the relationship between genetic diversity measured using RAPD markers with the magnitude of specific combining ability and heterosis.

## MATERIALS AND METHODS

### *Plant Materials:*

The present investigation was carried out at Agric. Res. and Experiment station, Faculty of Agriculture, Cairo University, Giza, Egypt, and Genetics and Cytology Department, National Research Center during 2007-2010. Three commercial cultivars, three newly developed lines and one introduced line of bread wheat (*Triticum aestivum* L. em. Thell) were kindly supplemented from Prof. Dr. M. A. El-Morshdey, Agronomy Department, Fac. Of Agric., Assiut Univ. and used as parental lines in this study; their pedigree and origin are given in Table (1). All possible cross combinations among parents, excluding reciprocals, were made to obtain twenty-one crosses. The twenty-eight entries, i.e., twenty-one $F_1$ crosses and seven parents were grown in a field evaluation experiment. The randomized complete block design (RCBD) with three replications was used. The experimental plot consisted of one row, 3m long and 30 cm wide with 15 cm between plants within rows. All other cultural practices were carried out according to recommendations.

### *Data Collected:*

Data were collected on days to 50% heading, plant height (cm), spike length (cm), number of spikes/plant, kernels weight/main spike (g), number of kernels/spike, 1000-kernel weight (g) and grain yield/plant (g).

### *Statistical Analysis:*

Data for all studied characters had been subjected to an ordinary analysis of variance according to Gomez and Gomez, (1984). The general (GCA) and specific (SCA) combining abilities were conducted according to Griffing (1956) method (П), model (I) (fixed effects). Better parent heterosis was calculated as the percentage of deviation of $F_1$ mean from the mean of the higher parent according to following formula:

$H = (\overline{F_1} - \overline{BP})/\overline{BP} \times 100$

**Table 1:** Pedigree and origin of the seven wheat parental genotypes used in the present investigation.

| Ent.No | Name | Pedigree | Origin |
|---|---|---|---|
| $P_1$ | Sids 4 | MAYA″S″/MON″S″/CMH74.A592 /3/GIZA 157*2 | Egypt |
| $P_2$ | Sakha 93 | SAKHA 92/TR 810328: | Egypt |
| $P_3$ | Gemmiza 9 | ALD″S″/HUAC ″S″// CMH74A 630/SX | Egypt |
| $P_4$ | Assiut 230 | (G 164x1228)(Kv2/Buha "s" Kal/Bb)x (134 x 5.69//86/3/386/7) | Egypt |
| $P_5$ | Assiut 249 | (G164x1204)=(Kv2/Buha "s" Kal/Bbx) | Egypt |
| $P_6$ | Assiut 216 | (562 x 1203)=(Local 2052 x 5500-10-21/29) 134 x 5.69-186/3/368/1 | Egypt |
| $P_7$ | Line 1457 | PI 3222 79 (Karchia) | India |

### *DNA Extraction and RAPD Amplification Conditions:*

Leaves were obtained from 14 days old plantlets and ground to a fine powder in liquid nitrogen. The genomic DNA was extracted using the BioFlux kit protocol. RAPD analysis was performed using eight 10-mer random primers (Table 2) produced from Operon Technologies (Metabion International AG).

RAPD assay was performed as described by Williams *et al.* (1990) with some modifications. PCR reaction was used in a final volume of 25 µl containing 12.5 µl of Master Mix (Bioteke), 2.5 µl of 5 µM of each primer, 50 ng of template DNA. PCR amplification was performed in PTC-100 PCR version 9.0 from M J Research-USA, programmed for 95°C for 5 min (denaturation), 36 cycles of {94°C for 1 min, 36°C for 1 min and 72°C for 1 min (annealing)} and a final extension of 2 min at 72°C. PCR products were analyzed using 1% agarose gel electrophoresis and visualized with ethidium bromide staining. The sizes of the fragments were estimated based on a DNA ladder of 100 bp (Fermentas).

**Table 2:** List of random primers and their nucleotide sequences used in RAPD analysis.

| Primer | Sequence |
|---|---|
| OPX-11 | 5́-GGAGCCTCAG-3́ |
| OPT-08 | 5́-AACGGCGACA-3́ |
| OPC-19 | 5́-GTTGCCAGCC-3́ |
| OPX-17 | 5́-GACACGGACC-3́ |
| OPD-13 | 5́-GGGGTGACGA-3́ |
| OPW-04 | 5́-CAGAAGCGGA-3́ |
| OPN-06 | 5́-GAGACGCACA-3́ |
| OPA-03 | 5́-AGTCAGCCAC-3́ |
| OPC-15 | 5́-GACGGATCAG-3́ |
| OPN-04 | 5́-GACCGACCCA-3́ |

*RAPD Data Analysis***:**

Clear and distinct amplification products were scored for presence (1), absence (0). The genetic similarity coefficient (GS) between two genotypes i and j was estimated using Dice coefficient (Sneath and Sokal, 1973). Dendrogram was built using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering procedure, based on the dissimilarity matrices obtained from RAPD data. Simple correlations were calculated between the genetic distance measured by RAPD marker with the amount of heterosis (H) and specific combining ability.

## RESULTS AND DISCUSSION

### 1. Field Experiment (Genetic Analysis of Wheat Characters):

### a. Analysis of variance:

The analysis of variance (Table 3) revealed that, mean squares due to genotypes, parents and $F_1$ crosses, were highly significant for all studied characters, indicating wide diversity among the parents. Also, mean squares due to parents vs. crosses, which are indication of the average heterosis, were found to be highly significant for all studied traits. Mean squares due to both general (GCA) and specific (SCA) combining abilities were highly significant for all studied characters. This indicated the role of both additive and non-additive types of gene effects in the genetic system controlling these characters.

**Table 3:** Mean squares for morphological and yield component traits of 28 wheat genotypes.

| Source of variation | d.f | M.S | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Heading date | Plant height | Spike length | No.spikes/plant | Kernel weight/main spike | No.kernels/ spike | 1000 kernel weight | Grain yield/plant |
| Rep. | 2 | 140.81 | 155.19 | 1.72 | 1.29 | 0.06 | 38.91 | 12.24 | 4.81 |
| Genotype | 27 | 86.93** | 533.88** | 11.46** | 21.63** | 0.97** | 360.48** | 45.74** | 197.1** |
| Parents | 6 | 188.87** | 456.3** | 16.39** | 21.0** | 1.54** | 727.11** | 22.02** | 149.94** |
| P vs. C | 1 | 66.04** | 1419.05** | 28.8** | 22.33** | 0.46** | 369.14** | 64.3** | 184.29** |
| Crosses | 20 | 57.38** | 512.88** | 9.12** | 21.79** | 0.82** | 250.05** | 51.94** | 211.89** |
| GCA | 6 | 363.78** | 1942.72** | 43.02** | 83.80** | 1.63** | 740.19** | 71.22** | 547.87** |
| SCA | 21 | 15.54** | 131.35** | 2.45** | 3.83** | 0.78** | 251.98** | 38.47** | 96.89** |
| Error | 54 | 3.48 | 7.50 | 0.26 | 0.69 | 0.0278 | 21.78 | 4.33 | 8.19 |
| GCA/SCA | - | 23.41 | 14.8 | 17.56 | 21.65 | 2.09 | 2.94 | 1.85 | 5.65 |

* and ** Significant at 0.05 and 0.01 probability level, respectively.

The ratio of GCA/SCA variances was more than unity for all the studied characters suggesting that additive were more important than the non-additive gene effects in the expression of these traits. These results are also in line with those obtained by Darwish *et al*. (2006), Motawea (2006), Koumber (2007), Akinci (2009) and Saeed *et al*. (2010). In addition, the magnitude of GCA mean squares was greater than SCA mean squares, suggesting that additive genetic effects were predominant and played a major role in the inheritance of all characters.

### b. Heterosis:

Heterosis recognized as the superiority in performance of hybrid individuals compared with their parents (Fehr, 1987). Better-parent heterosis is a comparison of the performance of a hybrid with that of its better parent. Desirable heterosis will be achieved when its value is positive for all studied characters, except days to heading that have a negative heterosis would be useful for earliness breeding.

As shown in Table (4), nine crosses had negative significant or highly significant heterotic effects for days to heading ranging from -4.14% for cross ($P_1xP_5$) to -12.01% for cross ($P_1xP_6$). The last cross also showed a positive highly significant heterosis value for grain yield (20.47) and may be recommended to be used in wheat breeding programs for improving both yield and earliness. For spike length, eight crosses had positive and highly significant heterotic effects, heterosis percentages relative to the better- parent ranged from -15.81% for the ($P_1xP_7$) cross to 16.13% for the ($P_3xP_6$) cross.

Estimates of heterosis for number of spikes/plant over the better-parent ranged from -33.33% for ($P_1xP_3$) hybrid to 18.18% for ($P_2xP_7$) and ($P_3xP_7$) hybrids. In addition, four crosses ($P_3xP_4$), ($P_4xP_6$), ($P_4xP_7$) and ($P_6xP_7$) showed positive and highly significant heterotic effects. Heterosis for Kernel weight/main spike ranged from -43.09% for the ($P_1xP_3$) cross to 20.18% for the ($P_4xP_7$) cross. Two crosses ($P_2xP_6$ and $P_4xP_7$) showed positive significant percentage of heterosis.

Heterosis in number of kernels/spike, ranged from -44.49% for the ($P_1xP_3$) cross to 23.68% for the ($P_3xP_7$) cross. Only one cross ($P_3xP_7$) showed positive highly significant percentage of heterosis. Estimates of heterosis for 1000- kernel weight ranged from -14.09% for the ($P_1xP_3$) cross to 16.27% for the ($P_4xP_7$) cross. Eight crosses showed significant or highly significant positive heterotic effects.

**Table 4:** Estimates of better-parent heterosis for the studied traits of 28 wheat genotypes grown under normal conditions.

| Crosses | Heading date | Spike length | No. spikes /plant | Kernel Weight/Main spike | No.kernels/ spike | 1000 kernel weight | Grain yield/plant |
|---|---|---|---|---|---|---|---|
| $P_1xP_2$ | -0.78 | 0.78 | -97.50** | -38.76** | -23.75** | 11.58** | -49.37** |
| $P_1xP_3$ | -6.74** | -4.81** | -33.33** | -43.09** | -44.49** | -14.09** | -53.13** |
| $P_1xP_4$ | -1.93 | 9.26** | -16.66** | -17.11** | -18.73** | -2.77 | -3.32 |
| $P_1xP_5$ | -4.14* | 2.22** | -33.29** | -11.13** | -12.38** | -7.76** | -9.03** |
| $P_1xP_6$ | -12.01** | -5.77** | -6.60** | -10.10** | -28.76** | -6.34** | 20.47** |
| $P_1xP_7$ | -9.59** | -15.81** | -27.27** | -20.00** | -27.76** | 1.98 | 11.55** |
| $P_2xP_3$ | -6.06** | 3.56** | -3.66** | -15.38** | -5.81 | 12.10** | -19.28** |
| $P_2xP_4$ | 4.22** | -5.84** | -4.13** | -15.68** | -16.39** | 10.98** | -19.69** |
| $P_2xP_5$ | 3.40* | -2.20** | -4.13** | -8.11** | -10.54** | 2.23 | -12.08** |
| $P_2xP_6$ | -0.60 | 5.30** | 0.00 | 2.16** | -6.25 | 9.67** | 2.36 |
| $P_2xP_7$ | 0.38 | -2.80** | 18.18** | -9.46** | -24.56** | 6.71** | 49.04** |
| $P_3xP_4$ | -5.05** | 4.65** | 11.11** | -29.49** | -34.87** | 5.00** | -21.65** |
| $P_3xP_5$ | -4.72** | -1.13** | -11.11** | -31.03** | -24.05** | 4.00* | -39.07** |
| $P_3xP_6$ | -2.02 | 16.13** | -14.77** | -4.10** | -13.99** | -10.97 | -19.43** |
| $P_3xP_7$ | -4.72** | -2.57** | 18.18** | -27.69** | 23.68** | 0.13 | 4.86* |
| $P_4xP_5$ | 5.66** | 10.30** | 0.00 | -7.31** | -28.99** | 2.23 | 2.08 |
| $P_4xP_6$ | -5.65** | 5.65** | 16.66** | -4.68** | -19.32** | -10.05** | 9.84** |
| $P_4xP_7$ | -2.94 | -1.63** | 09.09** | 20.18** | 1.73 | 16.27** | 78.05** |
| $P_5xP_6$ | -0.35 | -1.13** | -14.29** | 0.00 | -18.99** | -6.91** | -9.18** |
| $P_5xP_7$ | -1.47 | -13.33** | 0.00 | -4.91** | -18.57** | -2.70 | 23.84** |
| $P_6xP_7$ | -2.47 | -4.89** | 9.09** | -10.19** | -0.51 | 3.11 | 29.01** |
| L.S.D$_{0.05}$ | 3.04 | 0.83 | 1.36 | 0.27 | 7.62 | 3.39 | 4.67 |
| L.S.D$_{0.01}$ | 4.05 | 1.11 | 1.8 | 0.36 | 10.14 | 4.52 | 6.22 |

Heterosis percentages for grain yield/plant showed that positive and significant or highly significant heterosis values were obtained for eight crosses. Heterosis ranged from -53.13% for the ($P_1xP_3$) cross to 78.05% for the cross ($P_4xP_7$). The highest positive heterosis values were recorded for the crosses ($P_4xP_7$), ($P_2xP_7$), ($P_6xP_7$), ($P_5xP_7$) and ($P_1xP_6$) in this character. Several investigators found significant and positive heterosis (Ashoush *et al.,* 2001; Akhter *et al.,* 2003; El-Sayed and Moshref, 2005, Akinci 2009 and Kumar *et al.* 2011) for grain yield/plant. Our results showed that the maximum useful heterosis (78.05%) was observed in grain yield/plant for cross ($P_4xP_7$) followed by (49.04%) for cross ($P_2xP_7$) in the same character.

Positive and significant or highly significant heterotic effects for grain yield were associated with positive and highly significant heterosis for some yield contributing characters. For example, the cross ($P_4xP_7$) showed highly significant and positive heterosis for grain yield/plant along with high positive heterotic effects in number of spikes/plant, kernels weight/spike and 1000- kernel weight. This cross may be recommended to wheat breeding programs for improving above-mentioned traits. Another two crosses ($P_2xP_7$) and ($P_3xP_7$) showed heterosis in grain yield/plant that was associated with some agronomic traits, such as number of spikes/plant, kernels weight/spike, number of kernels/spike and 1000- kernels weight.

Akhter *et al.* (2003) found that positive and significant heterosis for number of spikes/plant, number of grains/spike, 100- grain weight and harvest index were frequently associated with significant heterosis for grain yield/plant. Shamsuddin (1999) reported a strong relationship between heterosis for grain yield and heterosis for some yield components. While, Prasad *et al*. (1998) reported that heterosis for number of grains/spike and 1000-grain weight was independently associated with heterosis for grain yield/plant.

### c. Combining Ability:

It is often desirable to select lines as parent of crosses. Most studies on wheat revealed that general combining ability (GCA) was found to be more important than specific combining ability (SCA) for number of spikes/plant. However, both additive (GCA) and non-additive (SCA) genes effects were observed for grain yield/plant, number of kernels/spike, 1000- kernels weight and number of tillers/plant. On the other hand, (El-Beially and El-Sayed 2002) concluded that mean squares associated with both GCA and SCA were significant for heading date, plant height, number of spikes/plant, number of kernels/spike, 1000- kernels weight and grain yield/plant.

Estimates of general and specific combining ability effects are presented in Table (5). For heading date, the parental genotypes ($P_1$) and ($P_2$) had highly significant negative GCA effects and may be considered as good combiners for earliness. While, in plant height, the parental genotypes ($P_1$), ($P_2$) and ($P_3$) showed highly significant and negative GCA effects, indicating that these genotypes have favorable genes for shortness. Specific combining ability for the $F_1$ crosses exhibited that none of the studied crosses showed significant and negative SCA effects for both days to heading and plant height.

The GCA effects for spike length showed that the genotypes ($P_1$), ($P_4$) and ($P_5$) had positive and highly significant GCA effects, while the remainder of parental genotypes had negative and highly significant GCA effects. Specific combining ability for the $F_1$ crosses showed that the crosses ($P_1xP_2$), ($P_3xP_4$), ($P_3xP_6$), ($P_4xP_5$),

($P_4$x$P_6$) and ($P_4$x$P_7$) had positive and highly significant SCA effects and could be considered as the best combinations for spike length.

For number of spikes/plant, the parental genotypes ($P_2$), ($P_3$) and ($P_7$) exhibited positive and highly significant GCA effects. The $F_1$ crosses ($P_2$x$P_7$), ($P_3$x$P_4$), ($P_4$x$P_7$) and ($P_6$x$P_7$) exhibited positive and highly significant SCA effects and could be considered as the best combinations for increasing number of spikes/plant.

**Table 5:** Estimates of general and specific combining ability effects for all studied traits of 28 wheat genotypes grown under normal conditions

| Parents & Crosses | Heading date (day) | Plant height | Spike length | No. spikes/plant | Kernel weight/main spike | No.kernels/ spike | 1000 kernels weight | Grain yield/plant |
|---|---|---|---|---|---|---|---|---|
| $P_1$ | -6.57** | -5.47** | 1.43** | -2.65** | 0.51** | 9.97** | 2.41** | -6.39** |
| $P_2$ | -0.68** | -8.58** | -0.51** | 0.32** | -0.01 | 0.75 | 1.22** | 1.58** |
| $P_3$ | 5.06** | -4.58** | -0.38** | 0.91** | -0.17** | -6.81** | -1.65** | 1.97** |
| $P_4$ | -0.53 | -0.28 | 1.57** | -0.27 | -0.02 | 0.71 | 0.77** | -0.42 |
| $P_5$ | 0.66* | 0.94* | 0.48** | -0.53** | -0.11** | 0.93 | -0.88** | -2.57** |
| $P_6$ | 2.21** | 0.46 | -0.58** | -0.87** | -0.04 | -1.92** | -2.09** | -2.22** |
| $P_7$ | -0.16 | 17.5** | -2.01** | 3.09** | -0.24** | -3.62** | 0.23 | 7.98** |
| $P_1$x$P_2$ | 1.23 | 3.68 | 0.88** | -0.56 | -0.96** | -3.75 | 6.53** | -5.95** |
| $P_1$x$P_3$ | 4.16** | 2.02 | 0.1 | -0.16 | -1.00** | 16.86** | -4.5** | -4.86** |
| $P_1$x$P_4$ | 2.42 | -1.61 | 0.46 | 0.03 | 0.1 | 1.29 | -0.79 | 0.75 |
| $P_1$x$P_5$ | 0.9 | 0.83 | 0.39 | -0.05 | 0.48** | 7.4** | -1.84 | 2.57 |
| $P_1$x$P_6$ | -2.32 | 1.98 | 0.12 | 0.29 | 0.38** | -6.08 | 0.14 | 2.43 |
| $P_1$x$P_7$ | -1.29 | 1.28 | -0.15 | -0.34 | 0.19 | -3.38 | -2.32 | 2.76 |
| $P_2$x$P_3$ | -1.06 | -1.87 | -0.13 | 0.45 | 0.06 | 7.36** | 2.99* | -1.60 |
| $P_2$x$P_4$ | 1.86 | -0.17 | -0.76** | -0.27 | -0.28** | -4.16 | 2.33 | -3.66 |
| $P_2$x$P_5$ | 1.68 | 0.61 | 0.00 | -0.01 | 0.09 | -0.05 | -0.25 | 0.59 |
| $P_2$x$P_6$ | 2.45 | 1.09 | 0.3 | 0.66 | 0.32** | 2.14 | 3.59** | 4.22** |
| $P_2$x$P_7$ | 1.82 | 3.72 | 0.66 | 1.69** | 0.18 | -9.82** | -0.82 | 6.88** |
| $P_3$x$P_4$ | -0.21 | 6.17** | 0.78** | 1.47** | -0.49** | -11.27** | 2.27 | -0.39 |
| $P_3$x$P_5$ | -1.06 | -2.39 | 0.04 | -0.27 | -0.45** | -3.16 | 3.49** | -3.96 |
| $P_3$x$P_6$ | 0.05 | 0.76 | 1.17** | -0.27 | 0.44** | 13.03** | -3.5** | 2.14 |
| $P_3$x$P_7$ | -0.25 | 11.06** | 0.2 | 1.10 | -0.19 | 11.07** | -1.08 | -0.06 |
| $P_4$x$P_5$ | 3.53** | 0.98 | 0.81** | -0.08 | -0.13 | -14.34** | 0.20 | -1.08 |
| $P_4$x$P_6$ | -2.36 | 1.13 | 1.14** | 0.25 | -0.19 | -3.82 | -4.69** | -0.81 |
| $P_4$x$P_7$ | -1.32 | 5.09** | 1.40** | 1.29** | 0.94** | 14.55** | 5.92** | 14.19** |
| $P_5$x$P_6$ | 1.45 | -1.43 | 0.24 | -0.49 | -0.10 | -4.05 | -1.44 | -1.55 |
| $P_5$x$P_7$ | -1.18 | 8.54** | -0.17 | 0.55 | 0.02 | -2.01 | -1.69 | 2.13 |
| $P_6$x$P_7$ | 0.27 | 8.35** | -0.44 | 1.88** | -0.31** | 0.51 | 1.42 | 3.21 |
| S.E$_{(gi)}$ | 0.33 | 0.48 | 0.09 | 0.15 | 0.03 | 0.83 | 0.37 | 0.51 |
| S.E$_{(sij)}$ | 0.97 | 1.41 | 0.26 | 0.43 | 0.09 | 2.42 | 1.08 | 1.48 |
| L.S.D$_{0.05(gi-gi)}$ | 0.65 | 0.94 | 0.18 | 0.29 | 0.06 | 1.63 | 0.72 | 0.1 |
| L.S.D. $_{0.01(gi-gi)}$ | 0.68 | 0.98 | 0.18 | 0.31 | 0.06 | 1.70 | 0.76 | 1.05 |
| L.S.D$_{0.05(sij-skl)}$ | 2.63 | 3.94 | 0.71 | 1.17 | 0.23 | 6.59 | 2.93 | 4.04 |
| L.S.D$_{0.01(sij-skl)}$ | 2.75 | 4.04 | 0.75 | 1.23 | 0.25 | 6.89 | 3.07 | 4.22 |

The general combining ability effects revealed that the parent ($P_1$) exhibited positive and highly significant GCA effects for kernels weight/main spike and number of kernels/spike, thus this genotype may be considered as a good source of genes for improving these traits. In addition, five and six crosses showed highly significant positive SCA effects for kernels weight/main spike and number of kernels/spike respectively.

For 1000- kernels weight, the parental genotypes ($P_1$), ($P_2$) and ($P_4$) exhibited positive and highly significant GCA effects, indicating that these parents are useful genotypes for improving this trait. Five $F_1$ crosses ($P_1$x$P_2$), ($P_2$x$P_3$), ($P_2$x$P_6$), ($P_3$x$P_5$) and ($P_4$x$P_7$) showed positive and significant or highly significant SCA effects, indicating that these crosses could be considered as the most promising for increasing 1000- kernels weight.

For grain yield/plant which considered as the most important trait, three parents ($P_2$), ($P_3$) and ($P_7$) exhibited positive and highly significant GCA effects reflecting that these genotypes may be considered as the best combiners for improving this trait The $F_1$ crosses ($P_2$x$P_6$), ($P_2$x$P_7$) and ($P_4$x$P_7$) showed positive and highly significant SCA effects, therefore, these crosses can be considered as the best combinations for increasing grain yield/plant. Similar results were obtained in wheat by Rasul *et al.* (2002), Singh and Singh (2003), El-Sayed (2004), Kamaluddin, *et al.* (2007), Salama (2007), Akbar *et al.* (2010) and Kumar *et al.* (2011).

One of the most promising crosses in this study ($P_4$x$P_7$) presented positive and highly significant SCA values for some important characters such as spike length, grain yield/plant, number of spikes/plant, kernels weight/spike, 1000- kernels weight and number of kernels/spike as well as insignificant value for days to heading. In addition, the hybrid ($P_2$x$P_6$) exhibited positive and highly significant SCA effects for grain yield, kernels weight/main spike and 1000- kernels weight.

These results suggested that the use of these crosses in breeding programs may help in producing promising pure lines with high yield.

*2- RAPD Banding Profile and Polymorphism:*

Results of DNA fingerprint revealed that the total number of amplified fragments differed among the primers used and ranged from six amplicons for the primers (OPW-04) and (OPN-06) to 10 amplicons for the primers (OPX-11) and (OPX-17). As shown in Table (6), the total number of DNA fragments amplified by all primers with all studied parents was 81 with an average of 8.1 amplicons/primer; among which, 50 amplicons were monomorphic with an average of 5.0 amplicons/primer, and 31 were polymorphic with an average of 3.1 amplicons/primer. Among the primers used, primer (OPX-11) amplified the highest number of monomorphic amplicons (9 amplicons), while primer (OPC-19) amplified the lowest number of monomorphic ampilcons (1 amplicon). Primer (OPC-19) amplified the highest number of polymorphic amplicons (8 amplicons), while, the primers (OPX-11), (OPW-04) and (OPA-03) amplified only one polymorphic amplicon.

Therefore, the different primers expressed different levels of polymorphism ranging from 10.00 % (primer OPX-11) to 88.89 % (primer OPC-19), with an average level of polymorphism of 37.64 % per primer. This average was lower than the earlier findings in wheat of Tahir (2008), Abd-El-Haleem *et al.* (2009), Nimbal *et al.* (2009), El-Assal and Gaber, (2012) which reached to 40.0 %, 70.5 %, 49.12 % and 52.0 % respectively. While, the results of El-Maghraby *et al.* (2010) in wheat is very close to our results (37.5 %). The size of amplified fragments varied with the different primers, ranging from 146 to 1363 bp (Fig. 1).

**Table 6:** Total number of amplicons, number of monomorphic and polymorphic amplicons and the percentage of polymorphism, as revealed by RAPD primers for the studied wheat parents.

| Primer | Total # of amplicons | # of mono amplicons | # of poly amplicons | Polymorphism (%) |
|---|---|---|---|---|
| OPX-11 | 10 | 9 | 1 | 10.00 |
| OPT-08 | 8 | 6 | 2 | 25.00 |
| OPC-19 | 9 | 1 | 8 | 88.89 |
| OPX-17 | 10 | 3 | 7 | 70.00 |
| OPD-13 | 7 | 5 | 2 | 28.52 |
| OPW-04 | 6 | 5 | 1 | 16.67 |
| OPN-06 | 6 | 3 | 3 | 50.00 |
| OPA-03 | 9 | 8 | 1 | 11.11 |
| OPC-15 | 9 | 6 | 3 | 33.33 |
| OPN-04 | 7 | 4 | 3 | 42.85 |
| Total | 81 | 50 | 31 | |
| Average | 8.1 | 5.0 | 3.1 | 37.64 % |

*a. Genetic Identification by Unique Markers for the Seven Parents:*

As shown in Table (7), the RAPD assay premitted the identification of five out of the seven parental genotypes by unique positive and/or negative markers. The five identified genotypes viz., Sids 4, Sakha 93, Gemmiza 9, Ass.249 and line 1457 were characterized by 13 negative and two positive unique markers. The genotype Sakha 93 was characterized by the highest number (5) of unique markers (4 negative and one positive); these unique fragments may be contributed in the shortness of this genotype. Sids 4 and Ass. 249 revealed four negative unique markers for each genotype. Sids 4 was carchterized by the lowest number of days to heading in our results, thus these unique bands may be responsible for earliness. Gemmiza 9 which carchterized by high yield and line 1457 which carchterized by the highest number of spikes per plant revealed only one unique marker each; positive for the first genotype and negative for the second. The other two parental genotypes ($P_4$ and $P_6$) did not exhibit any unique markers. Among the tested primers, two (OPT-08 and OPC-19) exhibited positive unique markers, while six (OPT-08, OPN-04, OPA-03, OPC-19, OPX-17 and OPN-06) revealed negative unique markers. The size of these unique markers ranged from 197 bp to 1180 bp.

*B. Genetic Relationships Between the Studied Parents as Revealed by Rapd Markers:*

The similarity indices estimates (Table 8) showed that the two most closely related cultivars were ($P_5$) Assiut 249 and ($P_6$) Assiut 216 with the highest similarity index (0.981). While, the most distantly related parents were ($P_2$) Sakha 93 and ($P_6$) Assiut 216, with the lowest similarity index (0.106).

On the other hand, the consensus tree (Fig. 2) was divided into two main clusters. The first cluster was further divided into two sub-clusters. One sub-cluster separated line 1457 ($P_7$), while the second sub-cluster included Assiut 249 and Assiut 216 ($P_5$ and $P_6$). The second main cluster was also divided into two sub-clusters; one sub-cluster separated the second parent (Sakha 93), while the other sub-cluster included the parental genotypes Assiut 230, Sids 4 and Gemmiza 9 ($P_4$, $P_1$ and $P_3$).
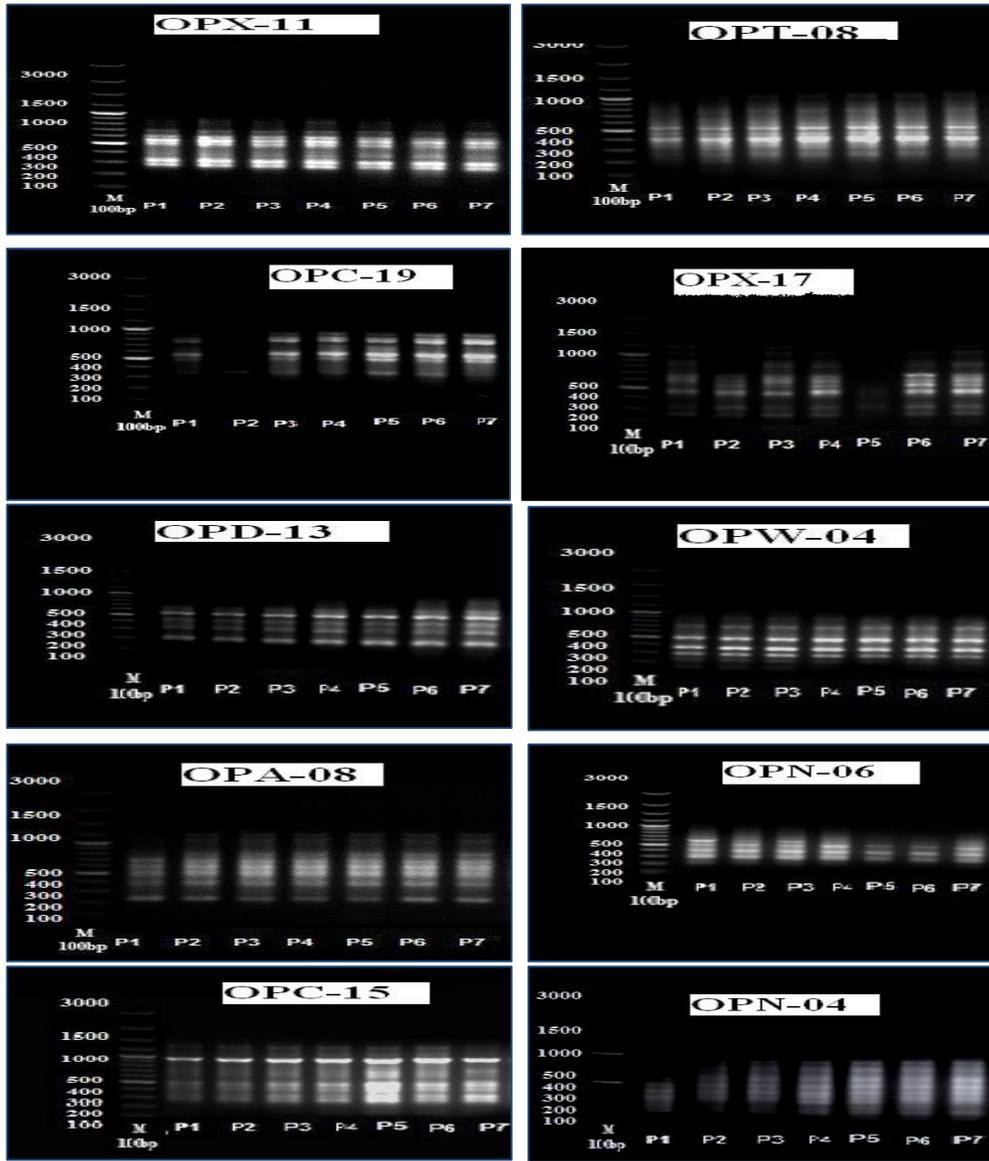
**Fig. 1:** RAPD profiles of the seven parental wheat genotypes amplified with primers OPX-11, OPT-08, OPC-19, OPX-17, OPD-13, OPW-04, OPA-08, OPN-06, OPC-15 and OPN-04.

**Table 7:** Positive and negative unique RAPD markers generated for five parental wheat genotypes.

| Genotype | Positive unique markers | | | Negative unique markers | | | Grand total |
|---|---|---|---|---|---|---|---|
| | Size/bp | Primer | | Size/bp | Primer | | 4 |
| P1 (Sids 4) | - | - | | 220 | OPT-08 | | |
| | - | - | | 536 | OPN-04 | | |
| | - | - | | 886 | OPN-04 | | |
| | - | - | | 1180 | OPA-03 | | |
| P2 (Sakha 93) | 252 | OPT-08 | | 730 | OPC-19 | | 5 |
| | - | - | | 645 | OPC-19 | | |
| | - | - | | 550 | OPC-19 | | |
| | - | - | | 500 | OPC-19 | | |
| P3 (Gemmiza 9) | 300 | OPC-19 | | - | - | | 1 |
| P5 (Ass. 249) | - | - | | 571 | OPX-17 | | 4 |
| | - | - | | 540 | OPX-17 | | |
| | - | - | | 268 | OPX-17 | | |
| | - | - | | 630 | OPN-06 | | |
| P7 (line 1457) | - | - | | 197 | OPW-04 | | 1 |
| Total | | | | | | | 15 |

**Table 8:** Genetic similarity matrices computed according to Dice Coefficient from RAPD between the seven parental wheat genotypes.

| Parents | Sids 4 | Sakha 93 | Gemmiza 9 | Ass. 230 | Ass. 249 | Ass. 216 | Line 1457 |
|---------|--------|----------|-----------|----------|----------|----------|-----------|
| Sids 4 | - | | | | | | |
| Sakha 93 | 0.572 | - | | | | | |
| Gemmiza 9 | 0.788 | 0.813 | - | | | | |
| Ass. 230 | 0.734 | 0.400 | 0.946 | - | | | |
| Ass. 249 | 0.277 | 0.411 | 0.462 | 0.600 | - | | |
| Ass. 216 | 0.510 | 0.106 | 0.146 | 0.814 | 0.981 | - | |
| Line 1457 | 0.344 | 0.257 | 0.184 | 0.907 | 0.475 | 0.907 | - |

Three groups were produced from the cluster analysis. The first group included three genotypes (Assiut 249, Assiut 216 and line 1457), of which Assiut 249 and Assiut 216 are closely related. The second group included (Sids 4, Gemmiza 9 and Assiut 230); within this category, Gemmiza 9 and Assiut 230 are closely related. The third group contained only one parent (Sakha 93).Information about genetic diversity of wheat genotypes is necessary for identifying diverse parental hybrid combinations that result in segregating progeny with high genetic variability for selection. In this respect, Sivolap *et al.* (1999) reported that random amplified polymorphic DNA (RAPD) technique has been successfully used for the assessment of genetic diveristy in diploid, tetraploid and hexaploid wheat.
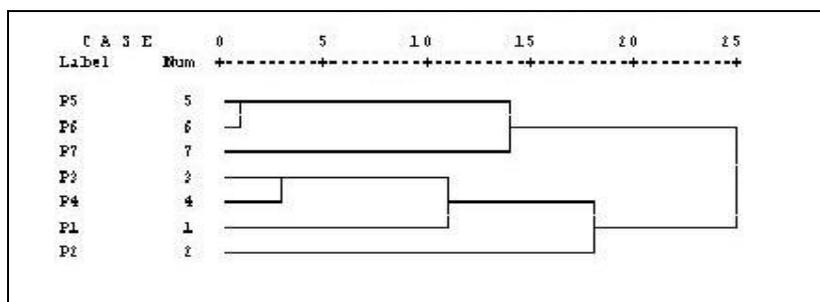


**Fig. 2:** Dendrogram for the seven parental wheat genotypes, constructed from RAPD analysis using unweighted pairgroup arithmetic average similarity matrix computed according to Dice coefficients. Where; 1=Sids 4, 2 =Sakha 93, 3=Gmmiza 9, 4 = Ass. 230, 5 = Ass. 249, 6= Ass. 216 and 7= Line 1457.

### C. The Relation Between RAPD Marker and Hybrid Performance for Grain Yield:

If a simple, efficient, inexpensive and reliable method could be used to predict heterosis before expensive field-testing, much of the field work associated with making crosses would be eliminate and hybrid breeding programs would be accelerated. For this reason, we focused on the relationship between heterosis and $F_1$ performance with the level of genetic diversity for parental genotypes measured by the RAPD marker for the most important character in wheat breeding programs i. e. grain yield/plant.

Non significant correlations were observed between genetic distance with both of the amount of heterosis (H) and SCA, as well as between heterosis with SCA (Table 9). Our results are in agreement with other results reported in wheat using RFLP (Corbellini *et al*. 2002) and RAPD (Perenzin *et al*. 1998) that could be attributed to the difference in the technique or to random primers used. Several previous findings (Zhao *et al.* 1999; Liu *et al.,* 1999; Bushehri *et al*., 2005 and Selvaraj *et al.,* 2010) reported that, due to complexity of genetical base of heterosis, it is hard to predict it from genetic distance. Therefore, there is no possibility to predict the hybrid performance through genetic distance.

**Table 9:** Pearson correlations between genetic distance (measured using Dice's coeffecient) with heterosis (H) and specific combining ability (SCA) for grain yield per plant.

| Parameter | Dice's GD | H | SCA |
|-----------|-----------|---|-----|
| Dice's GD | 1.000 | - | - |
| H | 0.0003 | 1.000 | - |
| SCA | 0.0056 | 0.127 | 1.000 |

** Correlation is significant at *P*=0.01

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