Heterosis in sunflower using cytoplasmic male sterility

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Abstract. A field trial was carried in the University of Anbar Alternation location in Iraq, the longitude 44° and latitude 33°. Eight cytoplasmic male sterile lines CMS, Female parents vis., A1, A3, A5, A11, and A21 and 3 tester restorers (Male parents which contains genes of fertility restoration and had mute- head (R-line) viz., (R1, R2, and R3) of sunflower were used in a Line x Tester mating system. In spring season 2018, to produce 15 hybrids. The genotypes seed 8 parents and 15 hybrids were planted in fall season 2018 using R.C.B.D. with three replicates. Analysis of variance revealed significant differences among the genotypes, parents, and hybrids in all studied characters. Parent A3 was disk area 194.31cm², seed number of disk 934.40 seed and seed yield per disk 67.73 gm, while the parent A11 gave high value for weight 1000 seed 74.2 gm, while the tester R1 gave high value for most of the characters. Hybrid A3 x R3 gave high value for head area 249.13 cm², for seed number of disk 1356.04 seed and seed yield per disk 94.71 gm, the hybrid A5 x R3 gave high heterosis for seed yield ratio 57.15%.

1. Introduction

The sunflower is one of the most important industrial crops in the world. It is ranked third after soybeans and rapeseed in the production of oil globally. Sunflower is considered one of the main sources of vegetable oil globally and the first oil crop in Iraq. And the second after the rapeseed in Europe [5], sunflower oil is good for containing the proportion of unsaturated fatty acids as well as good feed for the cows, animals, and poultry which are high protein content of 32% and carbohydrate 20-22% [8]. Despite the great importance of this crop, its productivity in the unit area is still low compared to global production. The average yield in Iraq is 2.11 tons.h⁻¹ while in the world is 7.5 tons.h⁻¹ [7]. Therefore, programs and plans should be developed to raise crop productivity and quality improvement.

The value of strains of hybrid sunflower in breeds and commercial values are depended on two factors. The first factor, characteristics of the same strains in the productivity of the yield crop and produce of sufficient quantities of pollen, and resistance to diseases and insects and environmental factors. The second factor is the behavior of the combining ability among strains in hybrids. The sunflower is cross pollinated crops, which is the yield will reduce in with internal mating and increases the value of yield when the mating happens with parents diverged genetically. Researchers produce single hybrids to achieve the maximum benefit from heterosis.

Single hybrid produces a higher yield as well as homogeneity of growth and maturity, the hybrid of sunflower cultivated more than 21 million hectares in the world [4], and that 100% of the production of sunflower oil in the United States of America, Europe, Argentina, and Australia is from hybrid farming. The discovery of Cytoplasmic Male Sterility (CMS) and Fertility Restorer genes (RF) by [13] in the development of the cultivation of the sunflower in the world. Hybrid production using cytoplasmic male sterility has become a necessity because of the nature of pollination in the crop, which requires the disposal of pollen for mothers for hybridization. Therefore, the hybrid sunflower breeding has...
progressed and developed rapidly and with high efficiency since applied techniques of male cytoplasmic sterility and fertility restorer genes.

The study aims are to evaluate the cytoplasmic sterility strains, fertile genetically, and to know the heterosis to select and produce the distinct hybrids and strains, also, of the studied traits.

2. Materials and Methods

A field study was conducted on five sterile strains of A-Lines and three fertility restoration R-lines. Consequently, the sunflower strains for hybrid production can be tested and evaluated by using cytoplasmic male sterility. These lines were obtained from the General Commission for Agricultural Research of Iraq. For hybridization according to the mating system (line x tester) Table 1.

| Number of lines | Code | Origin of line | Type of line |
|-----------------|------|----------------|--------------|
| 1               | R1   | Iraq           | R-Line       |
| 2               | R2   | Iraq           | R-Line       |
| 3               | R3   | Iraq           | R-Line       |
| 4               | A1   | Iraq           | A-Line       |
| 5               | A3   | Iraq           | A-Line       |
| 6               | A5   | Iraq           | A-Line       |
| 7               | A11  | Iraq           | A-Line       |
| 8               | A21  | Iraq           | A-Line       |

This experiment was conducted in the spring and fall seasons of 2018 in the fields of the College of Agriculture - the University of Anbar in Iraq, located at the longitude 44° and latitude 33°. The seed of strains for the spring season on 22/2/2018 was planted, such as one fertile line between two sterile lines with a distance of 0.25 m between plants and 0.75 m between lines. At the same place in randomized complete block design (RCBD) with three replicates. The sterile strains of cytoplasmic were fertilized using genetically modified strains using (line x tester) by (Kempthorne, 1957) to produce the seeds of the first-generation embryos and 15 hybrids. The lines (R1, R2, R3) were used male parents as the testers and the lines (A1, A3, A5, A11 and A21) as female parents. At the end of the season, the plants were harvested for each of the eight parents, and the 15 crosses were manually seeded for cultivation in the comparison experiment. In the fall season of 2018, hybrid seeds were planted with the parents on 25/7/2018 at the same place. In this study, the Randomized Complete Block Design (RCBD) was designed with three replicates. The experimental unit was consisting of 4 lines with a length of 5 m and a distance of 0.75 m between the lines and 0.20 m between the plants and the rate of 3 seeds in one place in the grand. After that, it diluted to one plant to obtain a plant density of 66666 plants. h-1. All agricultural operations were carried out according to the recommended recommendations. Add phosphate fertilizer 240 kg P2O5.-1h before planting. Add urea fertilizer at a rate of 280 kg N.-1h and in two increments [11]. The studied traits were determined on the basis of the individual plants and by ten plants. The guard lines were excluded, and the following traits were studied: plant height (cm), disk flower area (cm 2), number of seed disks, 1000 (gm) seed weight, plant seed yield (gm).

The data were statistically analyzed in the method of (Line x Tester) analysis proposed by [12] and explained by [21] in which the parents are divided into two groups. The parents used in the test are the testers, T = 3, and the parents to be evaluated, Lines, L = 5. The number of hybrids will be equal to the number of crosses. L × The number of T testers, which represents fifteen individual crosses. Therefore, the number of genotypes is 23 = (15 + 8).

The hybrid power of all studied traits was estimated from the mean of the replicates using the following equation

\[
\text{Best parent F1 Heterosis (\%H)} = \frac{F1 - BP}{BP} \times 100
\]
Heterobeltiosis = Heterosis $\sim F_1^+$ = First Generation Rate and Best Parent  

\(BP^+\): Best Parent Rate.

3. Results and Discussion

Table 2 shows the differences in the average squares of the genotypes, which were significant at 1% for all studied traits, as well as the averages of parent’s square and parents against hybrids, hybrids, lines, Tester, and the interaction between lines and testers. The interaction was significant for all studied traits. [1, 2, 3, 4, 8] showed that there were significant differences between genotypes, which allowed for further study of their genetic behavior.

Table 2: Analysis of variance in the (Line x Tester) method of the analysis of the studied traits in the sunflower:

| S.O.V                  | Degree of freedom (D.F.) | Mean Squares (M.S.) |
|------------------------|--------------------------|---------------------|
|                        | High plant (cm)          | Disk Area           | Number of Seed per Disk | Weight 1000 Seeds (gm) | Yield Seeds per Plant (gm) |
| Rep.                   | 2                        | 3.2                 | 11.2                     | 19.93                  | 48.3                        | 7.9                       |
| Genotype               | 22                       | 778.9\(^a\)        | 5993\(^a\)              | 83651.1\(^a\)          | 338.3\(^a\)                 | 848.2\(^a\)               |
| Parents                | 7                        | 899.0\(^a\)        | 8791\(^a\)              | 76896.2\(^a\)          | 468.91\(^a\)                | 1027.2\(^a\)              |
| Parents against hybrid | 1                        | 86.5\(^a\)         | 6131.2\(^a\)            | 141090\(^a\)           | 544.13\(^a\)                | 12.896\(^a\)              |
| Hybrid                 | 14                       | 68.5\(^a\)         | 232.7\(^a\)             | 9129.8\(^a\)           | 86.29\(^a\)                 | 17.79\(^a\)               |
| Lines                  | 4                        | 102.0\(^a\)        | 220.3\(^a\)             | 9657.4\(^a\)           | 88.02\(^a\)                 | 13.90\(^a\)               |
| Testers                | 2                        | 85.82\(^a\)        | 124.31\(^a\)            | 11115\(^a\)            | 100.85\(^a\)                | 16.08\(^a\)               |
| Line X Tester          | 8                        | 25.54\(^b\)        | 118.95\(^a\)            | 3078.9\(^a\)           | 31.02\(^a\)                 | 8.75\(^a\)                |
| Error                  | 44                       | 11.68               | 27.74                    | 64.23                  | 1.489                       | 2.82                      |

\(^a\)Significant at 1%  
\(^b\)Significant at 5%

Table 3 shows the average values of the parents and the individual hybrids resulting from the studied traits. There are significant differences between the genotypes and their individual crosses to the studied traits. Parent A3 is distinguished by giving the highest value in the disk area and the number of seeds by the disk and seed yield. It was 194.31 cm², 934.40 seeds, and 67.73 g respectively, parent A11 surpassed the weight of 100 seeds while parent A21 outperformed plant height while the parent A3 was superior to plant seed yield of 67.73 g. The hybrid 3R × A3 showed an increase of 249.13 cm² in the disk area, and the number of seeds with the disk 1356.04 seeds and plant seed yield was 87.71 g. This corresponds with the results of [9, 10, 11, 15, 16, 18] between individual hybrids and their parents for several studied traits.

Table 4 shows the heterosis of the individual hybrid in the studied traits. The genetic divergence between the breeds was reflected on the hybrid, and the heterosis attributed to the best parents differed. Differences in heterosis values were observed. It is positive in some hybrids and negative in other hybrids. Also, the table shows the hybrid strength desired for some traits. Five crosses gave heterosis positive strength of the height of the plant. In the disk area, hybrid 1R × A5 gave the highest positive hybrid strength of 52.27%, while the lowest in the hybrid 1R × A3 reached -5.91%. 1R × A11 hybrid gave positive heterosis for the number of seeds per disk and reached 96.94%. In contrast, the hybrid 2R × A5 in the seed weight gave the highest positive hybrid power 60.43%. In the yield of plant seeds, it showed 14 hybrids positive heterosis and significant, 3R × A5 and 1R × A11 showed the highest value for the strength of the hybrid reached 57.15% and 49.07% sequentially relative to the best parents. From this, it follows that the heterosis vary according to parents, and it is not necessary to get a hybrid high
with high quotient parents force any no fixed relationship between the quotient of grain for parents and 
the degree of heterosis hybrids produced them both, and this was supported by [5,6,14,17,19,20].

**Table 3:** Average studied traits of parents and first generation hybrids in sunflower plants.

| Genotypes | High plant (cm) | Disk Area | Number of Seed per Disk | Weight 1000 Seeds (gm) | Yield Seeds per Plant (gm) |
|------------|-----------------|-----------|-------------------------|-------------------------|----------------------------|
| 1R         | 124.13          | 78.31     | 610.13                  | 36.2                    | 21.89                      |
| 2R         | 111.06          | 80.84     | 496.50                  | 41.4                    | 19.78                      |
| 3R         | 99.39           | 85.20     | 469.30                  | 38.3                    | 20.56                      |
| A1         | 127.03          | 166.41    | 846.06                  | 65.5                    | 54.84                      |
| A3         | 135.12          | 194.31    | 934.40                  | 48.1                    | 67.73                      |
| A5         | 144.70          | 159.72    | 856.08                  | 60.4                    | 54.64                      |
| A11        | 140.30          | 162.98    | 634.40                  | 74.2                    | 59.07                      |
| A21        | 161.80          | 170.52    | 856.08                  | 62.5                    | 65.29                      |
| 1R × A1    | 145.15          | 169.41    | 740.16                  | 49.3                    | 65.22                      |
| 1R × A3    | 140.06          | 182.82    | 959.20                  | 42.6                    | 80.10                      |
| 1R × A5    | 152.80          | 243.21    | 1104.7                  | 64.7                    | 65.30                      |
| 1R × A11   | 132.68          | 231.96    | 1249.41                 | 59.5                    | 88.06                      |
| 1R × A21   | 164.51          | 184.71    | 1044.2                  | 84.2                    | 74.13                      |
| 2R × A1    | 118.20          | 161.05    | 900.87                  | 93.3                    | 66.50                      |
| 2R × A3    | 125.60          | 203.66    | 1011.19                 | 60.1                    | 59.33                      |
| 2R × A5    | 144.23          | 232.08    | 858.33                  | 96.9                    | 66.10                      |
| 2R × A11   | 125.18          | 199.49    | 987.16                  | 60.0                    | 77.74                      |
| 2R × A21   | 135.48          | 194.95    | 760.50                  | 76.6                    | 75.76                      |
| 3R × A1    | 118.65          | 218.98    | 1036.64                 | 84.2                    | 69.92                      |
| 3R × A3    | 124.00          | 249.13    | 1356.04                 | 69.2                    | 94.71                      |
| 3R × A5    | 136.81          | 210.62    | 1100.84                 | 69.7                    | 75.12                      |
| 3R × A11   | 137.60          | 191.05    | 1030.17                 | 87.8                    | 72.74                      |
| 3R × A21   | 161.45          | 221.69    | 1100.84                 | 87.8                    | 72.74                      |
| L.S.D 5%   | 4.60            | 7.09      | 10.79                   | 1.64                    | 2.27                       |

**Table 4:** Heterosis of the crosses relative to the best parents of the traits studied in sunflower:

| Genotypes | High plant (cm) | Disk Area | Number of Seed per Disk | Weight 1000 Seeds (gm) | Yield Seeds per Plant (gm) |
|------------|-----------------|-----------|-------------------------|-------------------------|----------------------------|
| 1R × A1    | 14.26           | 1.80      | -12.51                  | -24.73                  | 18.92                      |
| 1R × A3    | 3.65            | -5.91     | 2.65                    | -11.43                  | 18.26                      |
| 1R × A5    | 5.59            | 52.27     | 29.04                   | 7.12                    | 19.50                      |
| 1R × A11   | -5.43           | 42.32     | 96.94                   | -19.81                  | 49.07                      |
| 1R × A21   | 1.67            | 8.32      | -6.52                   | 34.72                   | 13.54                      |
| 2R × A1    | -6.95           | -3.22     | 6.47                    | 42.44                   | 21.26                      |
| 2R × A3    | -7.04           | 4.81      | 8.21                    | 24.94                   | -12.40                     |
| 2R × A5    | 0.32            | 45.30     | 0.26                    | 60.43                   | 20.97                      |
| 2R × A11   | -10.77          | 22.40     | 55.60                   | -19.13                  | 31.60                      |
| 2R × A21   | -16.26          | 14.32     | -11.16                  | 22.56                   | 16.03                      |
| 3R × A1    | -6.59           | 31.95     | 22.52                   | 31.90                   | 27.50                      |
| 3R × A3    | -8.23           | 28.21     | 45.12                   | 43.86                   | 39.82                      |
| 3R × A5    | -5.45           | 31.86     | 15.64                   | 7.45                    | 57.15                      |
| 3R × A11   | -1.92           | 17.22     | 19.87                   | -6.06                   | 27.17                      |
| 3R × A21   | 0.40            | 30.00     | 20.33                   | 40.48                   | 11.41                      |
| S.E        | 1.99            | 4.82      | 7.71                    | 7.26                    | 3.40                       |
4. Conclusion

The results show that the Heterosis Recurrent selection could be the best method to improve some of their inbreds, especially for having more variations among inbreds sand after selfing and selection for several generations. It was expected to have some elite hybrids.

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