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# UTILIZATION OF MALE STERILITY IN SUNFLOWER BREEDING FOR HETEROSIS

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The use of heterosis effect obtained in sunflower hybrids by crossing selected inbred lines is the most efficient method for further increase of oil production per unit area. Many scientists proved the considerably increased seed and oil yield per hectare in sunflower heterosis hybrids compared to variety-populations (Morozov, 1934; Iagodkin, 1937; Putt and Heiser, 1964; Gundaev, 1966; Volf, 1966; Vrânceanu and Stoenescu, 1972, etc). However, the introduction of hybrids displaying heterosis in agricultural practice appeared rather difficult in the last period of time because the genetic nature of male sterility was insufficiently investigated in sunflower. This at its turn hindered the investigations for elaborating a genetic system which should ensure to obtain commercial hybrid seeds (with a nearly 100% hybridity level).

French and Roumanian geneticists and breeders have great merits in working out these problems. Thus Leclercq in 1966 and Vrânceanu and Stoenescu in 1970 suggested a genetic system founded on the use of male sterility determined by genes for the practical breeding for heterosis. Due to the use of this system, in Romania were obtained and released for the first time in the world practice the single hybrids HS-53 and HS-52 which exceed by 24% the local variety Record with respect to oil yield per hectare.

Later on in 1969, following a cross between *Helianthus petiolaris* and *H. annuus*, Leclercq developed a source of cytoplasmic male sterility in sunflower. Most of lines and varieties crossed to this source proved to be sterility maintainers. Subsequent investigations carried out by the American, Canadian and Romanian scientists permitted to develop the first pollen fertility restorer sources and lines (Enns, et al., 1970; Kinman, 1970; Vrânceanu and Stoenescu, 1971).

There are therefore at the present time two genetic systems permitting to obtain commercial sunflower hybrids displaying heterosis.

Since 1967 the Unional Institute for Plant Breeding and Genetics has investigated the genetic nature of male sterility in sunflower. But among the examined variety-populations and lines of cultivated sunflowers no cytoplasmic male sterile forms could be discovered (Pogorletzki and Burlov, 1969, 1971). In our lines the male sterility character was in some cases controlled by a single gene and in others by recessive duplicate genes. No interaction was observed between these genes and the cytoplasm. In order to use the existent in our material male sterility it was necessary to employ the strong linkage between genes for anthocyanin colour and male fertility, descovered by Leclercq. Our investigations undertaken between 1968—1971 for getting such a linkage were successful (Burlov, 1972). But the female lines we obtained, with the purpose to produce commercial hybrids proved quite unsatisfactory with respect to the valuable economic characteristics. These hybrids were, as a rule, characterized by coarse husks and a low oil content. Since 1971 we have worked intensively for developing male sterile analogouses of the best male fertile lines, possessing high combining ability, and thus to obtain single heterosis hybrids.

In order to develop male sterile analogouses we used the $I_6$—$I_8$ lines *2586, 2625, 2589, 2600, 2621* and others, extracted from Armavirski 3497 variety, previously tested for their general and specific combining ability, with respect to the valuable economic features.

In 1971, green male sterile plants of the sterile source were crossed to the original male fertile line and parallelly the original male fertile line was crossed to the anthocyanin source. In November 1971, under greenhouse conditions, the male fertile hybrid heterozygous plants of the first crossing and the red male fertile plants of the second crossing were self-pollinated.

In May 1972 (second greenhouse generation), the progeny obtained from the first cross segregated in three green male fertile and one green male sterile phenotypical classes. The progeny of the second cross segregated in three red male fertile and one green male fertile phenotypical classes.

The green male sterile plants of the first type of crossing and the red male fertile plants of the second type were crossed by pairs and their hybrid seeds were sown in the field in July 1972. In August 1972, all red male fertile heterozygous plants (Tt Ms ms) were self-pollinated. In this way, about 150 plants were selfed for the male sterile analogous of the line No. 2586 and 50 plants for the line No. 2591.

In 1973, male sterile analogouses of the mentioned lines were obtained (5/8 of the genotype of the original line); they segregated 25% green male sterile plants and 75% male fertile plants and didn't differ phenotypically from the original lines.

Nevertheless these lines were very heterozygous for husk percentage and oil content in kernels and achenes (table 1).

Data presented in table 1 show that the male sterile analogouses were heterozygous for the main valuable economic character — the oil percentage in seeds. Thus, in line 2586 the weighted mean of this index

*Table 1*

**General characteristics of male sterile analogouses based upon their main valuable economic features (1973)**

| Investigated characters | Line number | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2586 ms | | | 2625 ms | | | 2599 ms | | |
| | Number of sublines examined | Extreme value of the character | $\overline{X} \pm t o5$ | Number of sublines examined | Extreme value of the character | $\overline{X} \pm t o5$ | Number of sublines examined | Extreme value of the character | $\overline{X} \pm t o5$ |
| 1000 seed weight (g) | 20 | 41—96 | 74±6.3 | 120 | 55—128 | 89 ±4.41 | 20 | 48 —107 | 70 ±2.19 |
| % husks | 20 | 41.6—25.0 | 34.6±2.25 | 131 | 39.3—24.1 | 30.4±0.59 | 20 | 37.8—25.8 | 30.9±1.0 |
| % oil in kernel | 20 | 52.6—62.7 | 57.9±1.46 | 88 | 46.4—62.6 | 54.7±0.65 | 20 | 41.8—62.7 | 55.8±2.0 |
| % oil in achene | 20 | 30.7—46.1 | 37.9±2.21 | 87 | 28.2—45.6 | 38.5±3.86 | 20 | 26.9—43.0 | 38.3±2.0 |

*Table 2*

**Caracteristics of the best sublines of the male sterile analogouses concerning their main valuable economic features (1973)**

| Line | index 1000 seed weight (g) | Husk % | Oil % in | |
|---|---|---|---|---|
| | | | Kernels | Seeds |
| 2586—246—15 | 68 | 26.5 | 62.7 | 46.1 |
| Armavirski 3497 | 92 | 23.9 | 61.3 | 46.7 |
| 2625—224—7 | 63 | 25.4 | 61.2 | 45.6 |
| Armavirski 3497 | 86 | 23.2 | 62.5 | 48.0 |
| 2599—242—2 | 61 | 26.2 | 58.2 | 43.0 |
| Armavirski 3497 | 89 | 23.6 | 64.7 | 49.4 |

was 37.9 $\pm$ 2.21% and the extreme values ranged from 30.7 to 46.1%. A similar aspect was also observed in other investigated lines and characters. The same phenomenon will be probably observed concerning the combining ability of these sublines. We shall get an answer to this problem in 1974, because, apart from the investigations regarding their economically valuable characteristics performed in 1973, all sublines were included in crosses with two testers, i.e. line 2586 and the variety Sputnik.

The main aim is the identification of the best sublines concerning their combining ability and the main valuable economic characteristics. The heterozygosity of these features allows the accomplishment of this task (table 2).

Data from table 2 show that the oil content in the kernels of the investigated sublines is close to the check, therefore the somewhat reduced oil content in sublines 2625—224—7 and 2599—242—2 may be explained to a greater extent by the higher husk percentage than by the smaller oil amount in kernels. In 1973, a second cycle of selection was performed within these and other sublines. With respect to these indices, we shall be probably able to separate male sterile lines similar to the open pollinated varieties.

A high heterozygosity was also observed in the male fertile lines ($I_5$—$I_{12}$) with respect to the husk and oil content in kernels and achenes. This may be explained by the fact that up to 1973 no breeding work was undertaken for these characters. For this reason the majority of single hybrids developed in 1972 and tested in 1973 were inferior to the cultivated variety Armavirski 3497 concerning the oil yield per hectare.

Parallelly to the selection of inbred lines for the main valuable economic characteristics a vast work is done to select them for general and specific combining ability. More than 200 male fertile lines ($I_4$—$I_5$) were evaluated in 1973 for their general combining ability. The test-hybrids were obtained in 1972 on two isolated fields. The inbred line 2586, outstanding for its high general and specific combining ability and the variety Peredovik, with a wide genetic base, were used as testers. In order to avoid the selfing within the inbred lines, the chemical emasculation with gibberellic acid was performed in the bud stage.

The hybrids obtained in this way were tested in 1973 on one row plots in three replications; the plot size was 6.4 sq.m. and the check variety Armavirski 3497 was sown every nine plots. The trials were performed in blocks formed by 23 hybrids and two checks. The estimation of the effects of the general combining ability was carried out according to Wolf's method.

Table 3 gives the effects of general combining ability of 18 male fertile lines remarkable for their oil production per hectare. First ranks the line 2608 in which the effects of general combining ability were high for all indices, including for oil yield per hectare. Line 136 proved also good, its general combining ability for oil yield per hectare being similar to that of line 2608.

*Table 3*

**Effects of general combining ability of the best male fertile lines with respect to the valuable economic characteristics (1973)**

| Line number | Yield | Husks | % Oil in | | Oil yield per hectare |
|---|---|---|---|---|---|
| | | | Kernels | Seeds | |
| 2608 | 6.5 | —0.96 | 2.9 | 2.7 | 3.73 |
| 136 | 7.0 | —4.1 | 3.4 | 4.9 | 3.61 |
| 2625—175 | 8.4 | 0.2* | 0.46* | 0.24* | 3.22 |
| 956 | 9.5 | 2.8 | 1.3 | —0.8 | 2.96 |
| 2586 | 5.8 | 0.39* | —0.3* | —0.4* | 2.38 |
| 448 | 3.5 | —2.5 | 0.3* | 1.8 | 1.81 |
| 383 | 3.8 | 1.13 | 0.8 | —0.64 | 1.78 |
| 2683 | 4.8 | 1.07 | —3.1 | —2.8 | 1.68 |
| 2625—3180 | 2.4 | 0.04* | 3.1 | 2.3 | 1.66 |
| 637 | 2.1 | —0 21* | 1.5 | 1.4 | 1.58 |
| 303 | 2.9 | —0.83 | 0.3* | 0.8 | 1.53 |
| 824 | 2.3 | —2.5 | 0.4* | 1.8 | 1.41 |
| 395 | 1.2 | —3.3 | 1.3 | 2.9 | 1.36 |
| 335 | 1.9 | —1.3 | —0.6 | 0.51* | 1.28 |
| 106 | 2.8 | 0.11* | 1.50 | 0.99 | 1.28 |
| 137 | 2.1 | —1.11 | 0.26* | 0.84 | 1.23 |
| 2625—404 | 3.6 | 0.35* | —0.43* | —0.46* | 1.21 |
| 402 | 0.7* | —2.8 | 0.5 | 1.01 | 1.00 |

*) nonconfirmed effects of general combining ability.

It is interesting to mention that the following three sublines: 2625—175, 2625—3180 and 265—404 ranked third, ninth and seventeenth respectively in what concerns the general combining ability effects for oil yield per hectare. Concerning seed yield, the subline 2625—175 ranked second in the respective group of lines; this proves that an effective selection may also be performed for this character.

28 direct hybrids, obtained by crossing 8 lines in a diallel system according to Griffing's fourth method, were tested in 1973. In 1972, parallelly to the 5 lines known for their combining ability the inbred lines 395, 400 and 394 with valuable economic indices, were included in the diallel crossing field. The hybrids obtained in 1973 were sown in three replications on randomized plots of 12.8 sq.m.

Table 4 gives the effects of the general combining ability and the variances of the specific combining ability of the investigated lines, concerning their valuable economic indices. The data of this table show that lines 2586 and 394 are the best with respect to the main valuable economic characters, having a high variance of the specific combining ability on the background of high effects of the general combining ability.

With respect to the specific combining ability the inbred line 2600 proved to be good, its combination 2599 × 2600 producing in 1972 a hybrid which exceeded by 25% the check with reference to the oil yield per hectare and these results were confirmed again in 1973; in 1974 this combination on male sterile basis is being tested in competitive trials.

*Table 4*

**Effects of general combining ability (gi) and variance of specific combining ability ($\Sigma^2$si) of inbred lines with respect to their main valuable economic characteristics (1973)**

| Inbred lines | gi | | | | | $\Sigma^2$ si | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Yield | Husks | % oil in kernel | % oil in achene | Oil yield | Yield | Husks | % oil in kernel | % oil in achene | Oil yield |
| 2013—395 | —5.5 | —1.88 | 0.21 | 1.29* | —1.83* | 30.7 | 0.91 | 6.41 | 3.22 | 3.5 |
| 2015—400 | 0.6 | —1.0 | 0.15 | 0.604* | 0.40* | 18.7* | 0.28 | 4.25 | 2.07 | 2.28 |
| 2009—394 | 4.0 | 0.57 | 0.96 | 0.39 | 1.45* | 52.6* | 0.51 | 5.12 | 3.16 | 7.02* |
| 3786—87—2658 | 1.72 | —0.18 | —1.10 | —0.81 | 0.37* | 26.3 | 0.53 | 2.68 | 0.83 | 3.26 |
| 590—2600 | —5.0 | 1.57 | —2.32 | —2.53 | —1.97* | 44.6 | 8.68 | 12.4 | 14.08 | 5.44* |
| 3761 (1002)—2599 | —2.1 | —0.88 | 1.85 | 1.84* | —0.40 | 19.9 | 10.17 | 5.37 | 9.94 | 3.44 |
| 3763 (1055)—2586 | 3.4 | 0.25 | 1.35 | 0.89 | 1.32* | 80.6 | 2.30 | 3.88 | 0.02 | 9.85* |
| 3771 (1057)—2610 | 3.0 | 1.57 | —1.08 | —1.66 | 0.67* | 15.7 | 3.84 | 3.40 | 2.82 | 2.14 |
| E dgi (±) | 0.27 | 0.3 | 0.40 | 0.42 | 0.127 | | | | | |
| E dgi-gi (±) | 0.23 | 0.46 | 0.12 | 0.648 | 0.191 | | | | | |
| | | | | | $\Sigma^2$ si | 36.1 | 3.40 | 5.44 | 5.16 | 4.62 |

* Effects and variances of general and specific combining ability nonconfirmed.

The selection for combining ability allowed to identify the best inbreds as concerns this characteristics, to transform them into male sterile lines and elaborate the crossing plan for 1974. In 1974 the best sublines of the male sterile analogouses 2586, 2625, 2600, 2599 and others were used as testers, while as male parents about 100 male fertile lines, with high general combining ability and valuable economic indices, were selected.

In 1973, during the examination of the male fertile lines for their combining ability nine single hybrids were identified which exceeded the variety Armavirski 3497 with respect to seed and oil yield, by 17—33% and 14—27% respectively (table 5).

The best hybrid in 1973 was 2625 × 2586 which exceeded the check Armavirski 3497 by 30% in seed yield and by 27% in oil yield. Two other hybrids, 260 × 2586 and 326 × 2586 were practically of the same value, their oil content in kernels being equal to that of the check variety and the husk percentage higher with 3.9—4.8%. The reduction of husk percentage in the parent lines will allow a further oil yield increase of these hybrids.

The mentioned hybrids are tried out again in 1974 on large plots in preliminary and competitive trials in two zones. Crossing fields were organized for these hybrids, using the male sterility linked with the normal colour of plants.

The following single hybrids obtained in Romania on the male sterility basis were tried out in 1973 : HS-52, HS-53, HS-61, HS-59, HS-40, HS-83 C and HS-81 C. Table 6 shows the characteristics of the

*Table 5*

**The main valuable characteristics of the prominent hybrids (1973)**

| Hybrids | Seed yield (q/ha) | % husks | Deviation from the check | | | Oil content in | | Oil yield (q/ha) | Deviation from the check for oil yield | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | yield (q/ha) | % | % husk | kernel | achene | | q/ha | % |
| 2625×2586 | 37.2 | 26.9 | +8.5 | +30 | +1.5 | 59.8 | 43.7 | 14.2 | 3.0 | 27 |
| 2599×2586 | 34.4 | 28.2 | +5.7 | +20 | +2.8 | 60.8 | 43.6 | 13.2 | 2.0 | 18 |
| 2610×2586 | 35.8 | 30.0 | +7.1 | +25 | +4.6 | 57,53 | 34.7 | 12.7 | 1.5 | 13 |
| Armavirski 3497 | 28.7 | 25.4 | — | — | — | 59.1 | 44.2 | 11.2 | | |
| LSD 0.95 | | | 4.3 | 18 | | P = 6.2% | | | | |
| 136×2586 | 36.7 | 25.7 | +5.3 | 17 | +1.4 | 63.2 | 47.0 | 15.2 | +2.4 | 19 |
| 956×2586 | 37.9 | 30.2 | +6.5 | 21 | +5.9 | 61.4 | 42.8 | 14.3 | +1.5 | 17 |
| Armavirski 3497 | 31.4 | 25.7 | — | — | — | 61.3 | 46.3 | 12.8 | | |
| LSD 0.05 | | | 2.8 | 9 | | P=1.04% | | | | |
| 260 ×2586 | 32.4 | 27.9 | +7.1 | +28 | +3.7 | 61.1 | 44.1 | 12.6 | +2.6 | 26 |
| 326 ×2586 | 33.8 | 28.8 | +8.5 | +33 | +4.8 | 58.4 | 11.9 | 12.5 | +2.5 | 25 |
| 2659×2586 | 30.4 | 26.5 | +5.1 | +20 | +2.1 | 60.1 | 44.4 | 11.8 | +1.8 | 18 |
| 2688×2586 | 33.8 | 30.2 | +8.5 | +33 | +6.2 | 55.5 | 38.4 | 11.4 | +1.4 | 14 |
| Armavirski 3497 | 25.3 | 24.0 | — | — | — | 59.4 | 45.1 | 10.0 | | |
| LSD 0.05 | | | 2.7 | 9 | | P=1.00% | | | | |

Romanian hybrids which gave remarkable results under climatic conditions prevailing in Odessa in 1973.

The above mentioned hybrids were tested in three replications on 12.8 sq.m. plots. The check was distributed after 9 numbers HS-59 and HS-61 were the best hybrids exceeding the check by 20—21% in oil yield. A decrease in husk percentage of the HS-61 parents will improve even more this hybrid in terms of oil yield per hectare. The positive aspect of the experimented hybrids originating from Romania (except

*Table 6*

**The main valuable economic characteristics of the Romanian sunflower hybrids (1973)**

| Hybrids | Seed yield (q/ha) | % husk | Deviation from the check | | | Oil content in | | Oil yield (q/ha) | Deviation from the check for oil yield | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | yield (q/ha) | % | husks | kernel | achene | | (q/ha) | % |
| HS — 61 | 31.2 | 28.7 | +6.6 | +27 | +2.7 | 62.8 | 44.7 | 12.3 | 2.1 | +210 |
| HS — 59 | 30.3 | 26.4 | +5.7 | +23 | +0.4 | 62.3 | 45.8 | 12.2 | 2.0 | + 20 |
| HS — 53 | 26.9 | 26.3 | +2.3 | + 1 | +0.3 | 63.8 | 47.0 | 11.1 | +0.9 | + 9 |
| HS — 52 | 23.9 | 25.7 | —0.7 | — 1 | —0.3 | 62.7 | 46.5 | 9.8 | —0.4 | — 4 |
| Armavirski 3497 | 24.6 | 26.0 | — | — | — | 64.0 | 47.3 | 10.2 | — | — |
| LSD 0.95 | | | 3.7 | 17 | | P = 5.6% | | | | |

HS-83 C) consists in their seed yield, combined with the reduced husk percentage and the high oil content. Taking into account this peculiarity, the Oil Crop Breeding Section of the Unional Institute for Genetics and Plant Breeding extracted inbred lines out of all these hybrids in 1973. By continually introducing the anthocyanin source in this material, male sterile lines will be developed as good female partners for crosses with the best male fertile lines, separated from the best domestic varieties, such as: Sputnik, Armavirski 14, Krasnodarski, Kruglik, etc.

At the Unional Institute for Genetics and Plant Breeding all hybrids were obtained by marked male sterility or by chemical emasculation with gibberellic acid. These two methods have their inconveniences especially with respect to hybrid seed production In the first case all red plants and the recombinations (green male fertile plants) must be discarded in crossing fields and in fields for increasing the female line during emergence-budding stage. In the second case, a chemical emasculation must be performed (at industrial scale) in crossing fields with the results depending to a great extent on the weather conditions prevailing at bud formation stage and also on a set of management problems.

It is beyond doubt that the utilization of cytoplasmic male sterile lines will remove all these difficulties of sunflower breeding and hybrid seed production. Bearing this in mind, the Unional Institute for Genetics and Plant Breeding started in 1973 working for the development of male sterile and fertility restorer analogouses of the best female and male parents of the hybrids with heterosis. In autumn 1974, the Institute will have at its disposal about 50 male sterile analogouses (BC-4) and 3—4 fertility restorer analogouses (BC-3 + BC-4) and the first single hybrids produced on cytoplasmic male sterility basis will be released in 1975.

We finally wish to stress that in the Oil Crop Breeding Section both genetically conditioned male sterility and the male sterility induced by gibberelic acid are used in practical breeding works for heterosis. We thus provide to a sufficient extent all the mechanisms that ensure a 100% hybridization of the parental inbred lines. However, we still have a small number of good lines with valuable economic characteristics with high effects of the general combining ability and with constant specific combining ability. Therefore, the main task for the present is to develop such inbred lines.

A wide mutual exchange of this type of lines among sunflower breeders will contribute to the rapid development and introduction in agricultural production of sunflower heterosis hybrids.