ADVANCES IN SUNFLOWER BREEDING IN ROMANIA

By

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Sunflower breeding at the Cereal and Industrial Crops Research Institute of Fundulea-Bucharest has been largely developed in the last 12 years, having as the main objects the increase of seed and oil yields; resistance to diseases; adaptability to different zones (especially to droughty areas); uniformity of height; flowering period and maturity. Although we have a large collection of sunflowers, the most valuable germplasm sources used for breeding purposes have been the Soviet VNIIMK varieties because of the low husk percentage and high oil content. Some Canadian entries containing genes for resistance to diseases have also been used. We have resorted to this collection more often in recent times, because we saw that we did not get the most satisfactory results regarding hybrid vigor by crossing only lines selected out of VNIIMK type varieties.

In the early years of our breeding program we focused our attention on creating sunflower varieties adapted to the soil and climatic conditions of Romania. The breeding material we started with was the valuable high oil varieties developed at Krasnodar by V. S. Pustovoit, and we also used his efficient breeding method. This resulted in some good selections, particularly regarding oil content. The high oil content variety Record was developed which is grown now on the whole area under sunflowers in Romania (10).

We ought to say that our attempt to increase simultaneously the seed yield and the oil content in seeds was not particularly successful. We realized that assured progress with respect to seed yield can be made only by using the heterosis effect that appears in the F_1 hybrids between inbred lines. It seems that even crossing among a limited number of inbred lines with high combining ability and high oil content in order to develop synthetic varieties would be a better way than the classic methods for variety improvement.

At the present time our breeding program is based exclusively on the complex selection of sunflower inbred lines and on genetic methods that will enable us to obtain the most efficient hybrids. The following are some aspects and results of this work:

Breeding for genetic and cytoplasmic male sterility. Genetic male sterility was noticed within many inbred lines and in various generations. In most cases a monogenic conditioning was revealed (9), and we have used for breeding purposes only those sources with a clear genetic and phenotypic expression.

Male sterility genes can be conditioned by various recessive genes, as was pointed out by Putt and Heiser (7), Gundaev (2), Leclercq (5), Anashchenko (1) and others. We have isolated more than 30 sources of male

sterility; ten of these being clearly conditioned by a single recessive gene. Diallel crosses between these ten male sterile lines (msms x Msms) indicated the presence of five different genes expressing the male sterile phenotypes. In Table 1 we present summarized data concerning lines which have one locus in common, showing in F_1 a typical 1:1 ratio. The male parents were always heterozygous plants, segregating in a 3:1 ratio.

There is no predictable relation between the genes for male-sterility and the sources they were isolated from, which proves their mutational origin. Three different genes (ms_1, ms_4, ms_5) were identified within VNIIMK 8931, while each of the first four genes are common for at least two varieties.

The multitude of genes for male sterility complicates to some extent the possibility of their utilization in producing hybrid seeds on the anthocyanic likage basis proposed by Leclercq (4). It is obvious that the linkage with the dominant gene T is obtained in one single case, when T-t and Ms-ms loci are situated in the same linkage group. Data from Table 2 shows that only ms_1 is linked with the anthocyanic gene since the progenies of the backcross ms_1ms_1 tt x Ms_1ms_1 Tt are segregating green-male sterile and anthocyanic-male fertile plants in a 1:1 ratio. In all the other cases, segregating ratios of 1:1:1:1 were obtained, with the four phenotypes in equal proportions (green-male sterile, anthocyanic-male sterile, green-male fertile, anthocyanic-male fertile). This proves that the genes ms_2, ms_3 ms_4, and ms_5 are not linked with the anthocyanic gene.

The anthocyanic source we have used in our work is the old Russian variety Fuksinka 3, which gives a close linkage with the ms_1 gene. We have had recombination rates ranging from 0.3 to 0.7% which is quite satisfactory for hybrid seed production. We do not know whether our anthocyanic gene is the same as the gene that Leclercq isolated from Nain-noir variety. It is very likely that there are more than one independent gene for anthocyanic pigment ans we have recently initiated testing of eight anthocyanic sources.

The identification of ms genes in sunflowers raised the question of relations between these genes and the cytoplasm and suggested the possibility of developing a genic-cytoplasmic system which would ensure completely male sterile progenies. With the purpose of detecting a fertilizing cytoplasm which would produce male fertile progenies in the presence of the recessive ms genes, as Hermsen(3) proposed for wheat and barley, we tested a large number of sunflower varieties with different genetical and geographic backgrounds. All of these sources of cytoplasm were crossed with heterozygous Msms forms as male parents, which always produced fertile F_1 generations.

In the F_2 901 progenies were analysed (Table 3). Both completely fertile and segregating progenies within each cross were noticed, their rate being close to a 1:1 ratio. Segregating progenies in F_2 conformed to a 3:1 ratio quite well.

Apart from varieties, we tested a large number of inbred lines, selected mainly from VNIIMK type varieties and never found a fertilizing cytoplasm. We can conclude, therefore, that the probability of finding a genetic-cytoplasmic system of male sterility in cultivated sunflowers is very low. Therefore, the cytoplasmic male sterility that Leclercq (6)

isolated by incorporating the H. annus genome into H. petiolaris cytoplasm seems to be the only real and available cytoplasmic male sterility source. In our breeding work this source gave completely male sterile progenies.

Breeding for combining ability. To establish inbred lines that give maximum heterosis when crossed is the main purpose in our sunflower breeding program. In comparison with the parental lines the heterosis of F_1 hybrids is very high, frequently exceeding 200%. However, for hybrid seed production to be profitable, we must also exceed the best varieties now grown. From this point of view, we have not yet obtained sunflower hybrids with spectacular yields. The seed yield of our best hybrid was 29% higher than that of the variety Record, but the frequency of such hybrids is low. From 950 hybrids we tested in the last three years, only 33 hybrids (or 3.4%) surpassed the seed yield of our best variety by 15-20%. One explanation of this low frequency of high yielding hybrids could be the fact that we have developed our lines by selfing almost exclusively within the VNIIMK type varieties which are closely related genetically.

The combining ability in sunflowers has to be tested both for seed yield and oil content in the seeds.

The oil content in seeds is determined by the relation between the husk percentage and the oil content in the kernel. These two components have a different heritability. The husk percentage has an intermediate expression in F_1; the value of the hybrids for this character depending directly on the parental inbred line value (Table 4). The oil content is a polygenic character with the dominant effects of gene action, which makes it possible to obtain hybrids with high oil content even if only one of the two parental lines has high oil content. The oil content of the kernel is also affected by heterosis. This is clear for all of the hybrids presented in Table 4, but especially for hybrids Nos. 1 and 3 which have 67.2 and 68.7% oil whereas their male parents have only 58.0 and 62.0% respectively. The female tester line had only 61.0%.

The general combining ability is determined by means of one monogenic male sterile line which is used as a female parent, and which has a sufficient number of male sterile plants to permit crossing under isolation with the inbred lines we have to test. We are testing annually 400 - 500 inbred lines in the 3rd or 4th generation. In the early generations selection for husk and oil content is accomplished. The inbred lines are classified in three categories according to the flowering date, each of them having one appropriate male sterile line as a tester. The specific combining ability is then determined by diallel crosses under isolation and artificial emasculation of female parents. The number of lines tested is limited to 10 - 15. The female lines of the best hybrids are backcrossed to the male sterile with anthocyanic linkage, because the probability that the initial line in which the ms gene was identified has a high combining ability is rather low.

Breeding for disease resistance. Downy Mildew (Plasmopara halstedii (Farl.) Berl. et de T.) and Sclerotinia are the most dangerous diseases of sunflower in Romania.

We have obtained good results by selecting for resistance to downy mildew within some Canadian cultivars. In 1966, we isolated the inbred line AD 66 out of the Canadian hybrid Advent (S37-388RR x Sunrise).

This line has shown absolute immunity to sunflower downy mildew when seedlings were infected with spore suspensions (Panchenko method), while other inbred lines tested simultaneously were 100% infected. A similar immunity was pointed out in 1969 in two Canadian inbred lines S37-388RR and CM 90RR containing single genes for rust resistance. This rust resistance gene, according to Putt and Sackton (8), was derived from a natural cross with Texas Wild Annual sunflower. It is very likely that the genes for rust and downy mildew resistance are located in the same linkage group, because these Canadian rust resistant lines, noted by RR, are at the same time resistant to downy mildew. On the other hand, the lines S37-388 and CM 90 are very susceptible to rust and are also very susceptible to downy mildew.

Crosses were carried out (12) between susceptible inbred lines and the immune line AD 66 in order to study the heredity of the resistance to downy mildew (Table 5). The F_1 hybrids were fully resistant to downy mildew both when AD 66 was the female and also when it was the male parent, which shows that no cytoplasmic factors are involved. In the reciprocal hybrid V3354 x AD 66 the two plants infected by Plasmopara were not hybrid; belonging to the female susceptible parent V3354. Dominance of resistance to downy mildew was complete in the F_1 generation. The F_2 progeny segregated in a clear ratio of 3 resistant plants to 1 susceptible plant. We have proposed the symbol Pl for the single dominant gene conditioning this resistance.

No genes for downy mildew resistance have been identified within VNIIMK type varieties, nor in other cultivars of the world collection. It is supposed, therefore, that the Pl gene, like the R gene, was derived from the Renner-Texas Wild Annual sunflowers. We started to transfer this gene into the male parent lines of the best single cross hybrids, which would enable us to grow hybrid sunflowers immune to downy mildew.

Breeding for the main agronomic characters. At present, we have more than 6000 inbred lines at our disposal, ranging from the first to the tenth selfing generation. In higher generations, there are homozygous inbred lines with a large variation of forms regarding stem height, length of growth season, head diameter and inclination, size and weight of seeds, husk percentage and oil content in the kernel. There are valuable inbred lines with low husk percentage (17-20%) and high oil content (60-64%) as well as dwarf and early lines, with large heads, resistant to drought.

Sunflower hybrid seed production. The use of the monogenic male sterility with anthocyanic linkage is at present the most feasible way to produce commercial hybrid seeds. There are no difficulties in organizing the hybrid seed production system; the area of crossing fields being at the most 1.5% of the whole area planned for sunflower production.

Great attention is being paid to the multiplication of parental lines, which is done on experimental farms. The male sterile market lines are grown under severe isolation (at least 3 km), seeding 2-3 seeds per hill. When the plants have two pairs of leaves, only one plant per hill is kept, by roguing anthocyanic plants from three-row bands alternating with one row from which, in turn, all green plants are rogued. In this manner, each three rows with green male sterile plants will be pollinated by one row with anthocyanic male fertile plants. At the beginning of flowering great care has to be taken in order to discard the green male

fertile plants which can occur as a result of recombinations. In order to maintain the male sterile line, only seeds from three-row bands with green male sterile plants are used.

The male parent lines are grown under 2 km isolation.

The crossing fields also have to be isolated at least 1.5 km. The ratio of the parental lines sown alternatively on these fields can be 4:2 or 6:2, depending on the pollen quantity the male parent produces and on the head size of the two parental lines. From this point of view it is desirable that the head diameter and flowering period of the male parent should be greater than that of the female parent. Flowering coincidence should be perfect or the male could even be 1-2 days earlier.

The female parent should be sown with a double or triple quantity of seed or with 2-3 seeds per hill. After emergence, all red-anthocyanic plants are rogued. A second roguing may be required later but before flowering, roguing all male fertile red or green plants that can occur on the female rows as well as the outcrossed plants from the male parent.

Ten single hybrids have been tested in comparative trials for three (1967-1969), three of them H552, H553 and H 555 giving the best results. They exceeded the standard variety by 24-29% for seed yield and 30-34% for oil yield per hectare, having an oil content in dry seeds of 50.5-52.5%. Apart from their productivity, sunflower hybrids have a high uniformity of height, head size and flowering and maturing period, which is very desirable for mechanized harvesting (11).

Sunflower hybrids will be available very soon for production purposes, contributing to the increase of the economic value of this important oil crop.

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Table 1. Diallel crosses between 10 monogenic male-sterile lines.

Crosses (ms ms x Ms ms)	Origin of female parent	Genes	No. of plants: Male-fertile	No. of plants: Male-sterile	Chi-square	P for fit to 1:1 ratio
AS 110 x AS 117	VNIIMK 8931	ms_1	86	92	0.20	0.50-0.75
AS 117 x AS 110	ARMAVIR 9345	"	96	104	0.32	0.50-0.75
AS 47 x AS 132	ARMAVIR 3497	ms_2	101	94	0.25	0.50-0.75
AS 132 x AS 47	VNIIMK 8931	"	94	98	0.08	0.75-0.90
AS 73 x AS 792	VNIIMK 8883	ms_3	98	107	0.37	0.50-0.75
AS 792 x AS 73	ENISEI	"	99	91	0.32	0.50-0.75
AS 77 x AS 403	VNIIMK 8883	ms_4	88	90	0.02	0.90
AS 403 x AS 77	VNIIMK 8931	"	97	87	0.53	0.30-0.50
AS 610 x AS 77	VNIIMK 8931	"	98	107	0.37	0.50-0.75
AS 77 x AS 610	VNIIMK 8883	"	104	96	0.32	0.50-0.75
AS 403 x AS 610	VNIIMK 8931	"	77	81	0.10	0.75
AS 610 x AS 403	VNIIMK 8931	"	88	99	0.64	0.30-0.50
AS 645 x ($Msms_1$ --- $Msms_4$)	VNIIMK 8931	ms_5	765	0	-	-

Table 2. Linkage between Ms-ms and T-t loci in sunflowers.

Crosses	Total plants	Observed ratio				Assumed ratio	P
		Green male-sterile	Green male-fertile	Red male-sterile	Red male-fertile		
ms_1ms_1 x Ms_1ms_1 Tt	92	43	0	0	49	1:1	0.50-0.70
ms_2ms_2 x Ms_2ms_2 Tt	83	19	22	24	18	1:1:1:1	0.70-0.90
ms_3ms_3 x Ms_3ms_3 Tt	124	33	27	29	35	1:1:1:1	0.70-0.90
ms_4ms_4 x Ms_4ms_4 Tt	117	25	32	26	34	1:1:1:1	0.50-0.70
ms_5ms_5 x Ms_5ms_5 Tt	95	19	26	21	29	1:1:1:1	0.30-0.50

Table 3. Sunflower cytoplasms tested in relation to ms genes.

No.	Tested cultivars (crossed by Msms)	No. of F2 progenies			Segregating F2 pooled ratio			
		Total	Non segregating	Segregating	Male-fertile	Male-sterile	P for fit to 3:1 ratio	Homogeneity (F test)
1	2	3	4	5	6	7	8	9
1	VNIIMK 8931	14	9	5	213	80	0.25-0.50	1.46
2	VNIIMK 8883	17	10	7	242	72	0.25-0.50	1.73
3	ZELENKA 368	17	8	9	311	101	0.75-0.90	0.61
4	ARMAVIR 3497	17	10	7	241	86	0.50-0.75	1.11
5	PEREDOVIC	16	8	8	245	92	0.25-0.50	1.86
6	SMENA	17	11	6	242	72	0.25-0.50	2.07
7	KRUGLIK A-41	12	8	4	168	62	0.25-0.50	2.17
8	SARATOV KARLIK	17	7	10	330	108	0.50-0.75	1.12
9	SARATOV 169	15	7	8	295	103	0.50-0.75	1.18
10	IRKUTSK	10	6	4	181	66	0.50-0.75	1.68
11	KARABALIKSKJ	10	7	3	147	43	0.25-0.50	2.49
12	KAMITINSKI 2	13	8	5	221	68	0.50-0.75	1.36
13	PIONER SIBIRI	12	5	7	252	75	0.25-0.50	1.10
14	STEPNEAK	16	10	6	243	79	0.75-0.90	0.49
15	BARNAUL 2151	11	4	7	214	60	0.25-0.50	1.91

Table 3 cont.

1	2	3	4	5	6	7	8	9
16	VORONEJ 109	14	7	7	235	68	0.25-0.50	1.75
17	PETROVSKI 2	10	7	3	161	50	0.50-0.75	2.11
18	FUKSINKA 3	17	8	9	311	94	0.25-0.50	1.68
19	JDANOV 6432	14	5	9	316	102	0.75-0.90	0.21
20	GIGANT 549	13	6	7	215	55	0.05-0.10	1.95
21	ZELENKA 61	12	8	4	192	48	0.05-0.10	2.28
22	ADVANCE	10	5	5	227	69	0.50-0.75	0.90
23	SUNRISE	11	5	6	264	96	0.25-0.50	1.26
24	COMMANDER	12	8	4	136	57	0.10-0.25	2.38
25	LAAN-PRETORIA	16	8	8	281	85	0.25-0.50	0.83
26	ELBA D. L.	16	6	10	320	116	0.25-0.50	0.71
27	JUPITER	14	10	4	177	51	0.25-0.50	2.25
28	COMET	12	8	4	188	55	0.25-0.50	1.86
29	GUNSON UNIV.	10	5	5	201	61	0.25-0.50	1.13
30	LANGESTEINNER	14	8	7	265	85	0.75-0.90	0.48

Table 3 cont.

1	2	3	4	5	6	7	8	9
31	OSTSONNE	11	5	6	248	66	0.10-0.25	2.10
32	BERNBURGERFUTTER	11	7	4	189	56	0.25-0.50	2.38
33	SOFIA N-85	14	10	4	165	64	0.25-0.50	2.21
34	MEZEHEDESHY	13	7	6	219	65	0.25-0.50	1.62
35	MESTEN OREAHOVKA	10	3	7	241	96	0.10-0.25	1.83
36	SLOVENSKA SIVA	10	4	6	197	70	0.50-0.75	0.42
37	DISCOVOLANTE	17	7	10	332	126	0.10-0.25	1.73
38	HYBRID MINNESOTA	14	7	7	240	81	0.75-0.90	0.12
39	KLEIN	14	9	5	198	54	0.10-0.25	1.96
40	MARAVILLA GIRASOL	13	5	8	268	100	0.25-0.50	0.72

Table 4. General combining ability of some inbred lines (crosses with monogenic female tester AS 110).

Male parent	Seed yield (Q/ha)			Husks (%)			Oil in kernel (%)		
	♀	♂	F_1	♀	♂	F_1	♀	♂	F_1
S1358	14.3	14.7	31.8	25.1	19.2	22.8	61.0	58.0	67.2
P1380	14.3	15.1	31.3	25.1	19.4	21.6	61.0	57.4	66.7
V1303	14.3	11.8	23.9	25.1	22.6	23.8	61.0	62.6	68.7
SR1415	14.3	15.7	22.5	25.1	43.9	35.0	61.0	58.8	65.5
BR1422	14.3	19.3	21.8	25.1	31.0	27.0	61.0	58.5	64.1
HT306	14.3	5.7	21.4	25.1	22.6	23.6	61.0	48.0	64.7
P1385	14.3	10.7	19.3	25.1	21.7	24.0	61.0	58.3	67.4
LN1393	14.3	8.6	16.8	25.1	49.0	44.1	61.0	51.7	62.6
V3280	14.3	13.6	16.4	25.1	26.7	25.3	61.0	61.8	65.0
A2-2750	14.3	7.1	13.6	25.1	23.9	24.6	61.0	53.8	62.7
A2-2740	14.3	12.3	13.4	25.1	25.9	26.0	61.0	59.8	62.0

Table 5. Segregation of resistance to downy mildew in sunflowers

Crosses and parental lines	F_1 generation			F_2 generation			
	Non infected	Infected	Plants infected %	Observed non-infected:infected	Segregating ratio	Chi-square	P
AD66	92	0	0	120: 0			
V3354	0	142	100	0:117			
AD66 x V3354	134	0	0	139:42	3:1	0.311	0.50-0.75
V3354 x AD66	141	2	1.4	126:39	3:1	0.164	0.50-0.75
AD66	108	0	0	104: 0			
S1366	0	121	100	0:128			
AD66 x S1366	115	0	0	116:41	3:1	0.104	0.75-0.50
S1366 x AD66	134	0	0	123:44	3:1	0.161	0.75-0.50