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RESISTANCE TO DOWNY MILDEW OF SUNFLOWER HYBRIDS BRED ON THE CMS BASIS WITH RESTORED POLLEN FERTILITY

Sunflower breeding on the basis of male sterility is a most effective method of obtaining the heterosis effect and using it in production.

Sunflower male sterility was discovered by Kuptsov (USSR) in 1935, but it has been intensively studied only during the recent 15 to 20 years. Works published in various countries reported that sunflower male sterility was found and that this was highly significant; such works were published in Romania (Vulpe, 1964), the USSR (A.I. Gundayev, 1967), France (P. Leclercq, 1966), Canada (E.D. Putt and Heiser, 1966), and Bulgaria (N. Stoyanova, 1967).

The practical results obtained in 1965-1966 (Vulpe, 1967) showed the economic significance of the sunflower hybrids bred on the basis of male sterility. At the first stage several simple hybrids were tested experimentally and in production with the employment of lines genetically marked by anthosyan, and it was believed that sunflower hybrid seeds could be definitely obtained. However, it was found at a later stage that the pollination of sibs can be completely dispensed with both on the breeding ground where genetically marked lines are generated and in the hybridization breeding grounds. Hybridization is incomplete because it is impossible fully to remove recombinants from the hybridization plots before their pollen leaves the pollen sacs. As a result production crops may include, apart from hybrid plants, also typically maternal line specimens which impair the yield. It was found that the defects of sunflower hybrid seed-growing can be only eliminated by using maternal lines possessing cytoplasmic

male sterility in combination with paternal forms of pollen fertility restorers.

A breakthrough was made in this field too. In Romania, for example, genotypes were found with genome SRfrf in the VNIIMK 8931 variety imported in 1956, which served as initial material for obtaining samples with male sterile cytoplasm and fertility restorers. Moreover, forms with cytoplasmic male sterility were obtained by crossing the species Helianthus rigidus with the species Helianthus annuus (1964) and subsequently by crossing the species Helianthus petiolaris (of the Argentine origin) with the Helianthus annuus. All the three sources belong to the B type of male sterility (Vulpe, 1968, 1974).

In France, a form possessing cytoplasmic male sterility was also obtained by crossing Helianthus petiolaris with the Helianthus annuus (Leclercq, 1969).

After the discovery of fertility restoring genes in (Rf) it was found that there is a double control of male sterility character, determined by the interaction of nuclear factors with sterile cytoplasm. In this case male sterility is cytoplasmic and genetically determined (i. e. nuclear cytoplasmic) (Vulpe, 1968). Since the term "cytoplasmic male sterility" is in wide use in practice we consider it possible to use it conventionally in the case of sunflower just as in the cases of maize, sugar-beet, wheat, sorgo and other crops, meaning by this a double control of the heritability of this phenomenon.

This paper gives the results of experiments with sunflower hybrids (both regionalized and experimental) the seeds of which are obtained on the basis of lines with cytoplasmic male sterility, and the fertility restorer lines resistant to downy mildew (Plasmopara helianthi).

The Material and Methods Used

In the period 1973-1975, in the climatic conditions favourable for the sunflower culture (ex-

cept the less favourable years 1968 and 1973). the Research Grain and Industrial Crops Institute conducted comparative variety testing to study different sunflower hybrids selected on the basis of male sterility.

The simple hybrids selected include above all the regionalized hybrids HS90 (Romsun 90) and HS301 (Romsun 301) as well as the experimental hybrids HS305, HS308 and HS310.

The HS305 hybrid which during 1968-1973 was known as HS72 is the first sunflower hybrid bred in Romania on the basis of male sterility (Vulpe, 1967, 1971).

The HS90 and HS301 are simple regionalized hybrids studied in comparative variety testing beginning with 1966.

The HS308 and HS310 are new simple sunflower hybrids tested during the two last years and created on the basis of cytoplasmic male sterility.

Up to 1972 the seeds of the HS305, HS90 and HS301 hybrids were grown on the basis of nuclear male sterility with the use of genetic markers, but subsequently they were transferred onto the cytoplasmic basis.

To transform nuclear male sterility lines into cytoplasmic male sterile lines we selected recombinants (green fertile plants) within the maternal line, which were later inbred and transferred onto the cytoplasmic basis with the help of backcrosses. Following six consecutive backcrosses the normal inbred lines, regardless of their origins, became cytoplasmic male sterile lines with the genome of the recurrent parent being inoculated into the donor's unchanged cytoplasm (sterile cytoplasm of the Petiolaris type produced in Romania).

Fertility restorer genes were transferred to the normal inbred lines by a preliminary conversion of an appropriate line into the cytoplasmic male sterile one (having the annuus-type cytoplasm obtained in Romania within the framework of the VNIIMK 8931 variety). The line was

then crossed with the Rf donor and the fertile castrated plants were subsequently backcrossed with the fertile analogue which did not restore fertility. After four consecutive backcrosses the fertility restorer lines were homozigated by self-pollination coupled with genetic selection.

As regards resistance to downy mildew (Plasmopara helianthi) we used the Canadian line S-37-388RR as the source of resistance for one of the parent lines. The Canadian line has the pleotrope gene R_1 which guarantees resistance both to rust (Puccinia helianthi) and to the European race of downy mildew; for the second parent line we used the American line HA-61 which has a gene securing resistance to the American downy mildew race Red river.

The two genes - R_1 and H, one from each line of the simple hybrid, - were transferred by crossing them with the donor used as the paternal form, which was followed by three or four consecutive crossings with the recurrent parent. This was culminated in the homozygotization of the lines through inbreeding coupled with genetic selection. Appropriate conditions were provided for the artificial inoculation by the European race of downy mildew. The H gene was transferred to the bulk of fertile analogues (B lines) of the cytoplasmic male sterile lines (A lines) so as to turn the latter into the homozygous lines as continuous backcrosses were conducted.

Beginning with 1973 hybrid seeds were produced for all the five variants on the basis of cytoplasmic male sterility, restoration of pollen fertility and the resistance to downy mildew (Plasmopara helianthi).

Hybrids were tested by the method of the Latin quadrangle with eight variants, in four replications and with the varied location of plots (V. Vulpe, 1967). The results were processed by the dispersion analysis.

Between 1965 and 1968 the increments in the seed and oil yields were expressed as percentages of the VNIIMK 8931 variety and beginning with 1968 in relation to the Rekord variety.

Phenological observations, biometrical measurements and laboratory seed analyses were conducted in all the experiments.

The Results Obtained

Plant growth. Simple sunflower hybrids are more uniform and powerful than the Rekord variety plants. They grow intensively just after the appearance of shoots with the early ripening and semi-early hybrids being most pronounced in this respect.

Restoration of pollen fertility. During flowering it was found that in all the five hybrids in P_1 fertility is restored completely, with natural pollination occurring in normal conditions.

The vegetation period of the hybrids under study is basically shorter than in the check variety (Table 1). The simple hybrid HS90 is the most early ripening one.

The height of plants. With the exception of the HS310 hybrid the height of the hybrid plants was less than that of check plants, the HS90 being the shortest. The hybrids' uniform height can be authentically illustrated by the variability coefficient of this feature.

The head diameter is larger in hybrids than in the check with the variability coefficient showing a greater uniformity of hybrids.

The thousand-seed and natural weights vacillated depending on the hybrid, but they were higher in hybrids than in the check variety.

Resistance to downy mildew (Plasmopara helianthi). Experiments have shown that there is a considerable difference between the resistance of hybrids and their parental forms to the European race of downy mildew and their sensitivity to it (Table 2).

Table 1

Characteristics of Some of the Sunflower Simple Hybrids

Variant	Period	Height		Head diameter		Thousand seed weight, g	Natural weight, kg	Vegetation period as percentage of the check, +
		cm	S %	cm	S %			
1	2	3	4	5	6	7	8	9
VNIIMK 8931 (check)	1965-1968	194	8.3	18.8	11.4	63.8	41.9	125
Record (check)	1969-1975	210	9.7	20.6	11.6	63.5	40.6	128
HS305	1965-1968	143	4.6	19.1	8.1	65.7	45.1	-21
	1969-1975	166	4.4	22.3	8.2	67.9	43.4	-11

Table 1 (cont.)

1	2	3	4	5	6	7	8	9
HS 90	1966-1968	141	4.5	18.9	8.8	64.7	43.9	-9
	1969-1975	136	4.3	22.2	8.6	67.3	41.4	-10
HS301	1966-1969	158	5.0	21.5	8.9	64.8	42.0	-3
HS308	1975-1974	167	4.8	22.1	8.4	69.3	41.4	-6
HS310	1974-1975	190	4.6	22.9	8.3	68.0	42.5	-5

Table 2

Resistance of Some of Simple Sunflower Hybrids and Their Parental Forms to Downy Mildew (Plasmopara Helianthi)

Initial variants	Frequency of occurrence			Intensity of occurence			Degree of occurence		
	♀	♂	♀×♂	♀	♂	♀×♂	♀	♂	♀×♂
Record (check)		100			100			100	
HS305	100	0	0	100	0	0	100	0	0
HS90	100	0	0	100	0	0	100	0	0
	0	100	0	0	100	0	0	100	0
HS301	100	0	0	100	0	0	100	0	0
HS308	100	0	0	100	0	0	100	0	0
HS310	100	0	0	100	0	0	100	0	0

The figures cited in Table 2 show that all the five simple hybrids proved resistant to the European race of downy mildew, with the exception of the Rekord variety and the parental forms devoid of the R_1 genes which were infested completely. The two variants of the HS90 hybrid show that the simple sunflower hybrid can be resistant to downy mildew in P_1 if one of the parental forms (no matter which) is homozygously resistant to that disease. It has thus been confirmed that sunflower genetic stability to mildew is dominant and monogenously conditioned.

To make the hybrids resistant also to the American race of downy mildew one of the paternal forms was supplied with the H gene so that, beginning with 1977, the seeds of the HS90 and HS301 hybrids will be sown on the basis of cytoplasmic male sterility and the resistance to both the European and the American mildew races.

Yield. Both the seed and oil yield of the simple sunflower hybrids studied in competitive testing without irrigation fluctuated depending on the year (Table 3).

From the figures in Table 3 one can see the marked advantages of simple hybrids over the check Rekord variety. The yields were high during several years of testing, yet they differed depending on the climatic conditions of each year. The annual fluctuations of the seed and oil yields of simple hybrids as compared to the Rekord variety are explained by a greater ecological plasticity of the Rekord variety's polyhybrid population than is the case of simple hybrids whose narrow hereditary basis accounts for a weaker ecological plasticity and consequently a certain instability in relation to the changed external factors.

To make a full use of the biological ability of simple sunflower hybrids to give big yields it is therefore necessary to apply the requisite agronomy to each simple hybrid both with and without irrigation (Vulpe, 1971, 1972).

Table 3

Fields of Some of the Simple Sunflower Hybrids

Variant	Year	Seed yield			Oil yield			Husk-ness, %	Percentage of oil in seeds, %
		c/ha	%	degree of authenticity	c/ha	%	degree of authenticity		
1	2	3	4	5	6	7	8	9	10
VNIIMK 8931 (check)	1965	35.1	100.00		15.2	100.00		24.2	49.1
	1966	29.1	100.00		12.8	100.00		23.6	50.0
	1967	33.7	100.00		14.5	100.00		24.2	48.9
	1968	31.7	100.00		13.8	100.00		23.7	49.2
	M	32.4	100.00		14.1	100.00		23.9	49.3
Record (check)	1969	22.8	100.00		9.8	100.00		23.00	48.9
	1970	33.2	100.00		14.6	100.00		22.3	50.1
	1971	33.6	100.00		13.7	100.00		22.6	47.2
	1972	34.3	100.00		14.5	100.00		23.3	48.1
	1973	33.8	100.00		14.8	100.00		23.7	49.7

Table 3 (cont.)

1	2	3	4	5	6	7	8	9	10
	1974	41.6	100.00		17.2	100.00		21.7	49.0
	1975	37.7	100.00		15.8	100.00		21.2	47.6
	M	33.8	100.00		14.3	100.00		22.5	48.6
HS305	1965	36.7	104.56		15.8	103.94		25.0	49.0
	1966	34.1	117.18	**	14.5	112.50	*	24.2	48.2
	1967	36.1	107.12		15.3	105.52		24.8	48.1
	1968	30.7	96.84		13.2	95.65		25.0	48.9
	M	34.4	106.17		14.7	104.25		24.7	48.5
	1969	24.7	108.33	*	10.2	104.08		24.7	47.1
	1970	39.1	117.77	**	17.5	119.86	***	20.2	50.8
	1971	36.8	109.52	*	16.2	118.25	***	20.1	49.9
	1972	40.6	118.37	***	18.8	129.65	***	20.3	49.2
	1973	35.0	103.55		16.0	108.11		20.2	52.2
	1974	46.6	112.02	*	19.7	114.53	*	21.3	49.3

Table 3 (cont.)

1	2	3	4	5	6	7	8	9	10
	1975	45.6	121.28	***	19.6	126.82	***	21.5	49.7
	M	38.3	113.31	*	16.9	118.18	**	21.2	49.7
	1966	34.9	119.93	***	15.3	119.53	***	24.0	49.8
	1967	36.9	109.49	*	15.8	108.96	*	24.2	48.6
	1968	31.1	98.10		13.4	97.10		23.8	49.0
HS90	M	34.3	108.88	*	14.8	108.03	*	24.0	49.1
	1969	26.9	117.98	**	10.6	108.16	*	25.5	46.6
	1970	39.9	120.18	***	17.9	122.60	***	20.0	50.6
	1971	37.0	110.12	*	16.5	120.44	***	19.4	50.7
	1972	34.1	99.42		15.0	103.45		22.6	49.8
	1973	36.2	107.10	*	16.7	112.83	*	18.3	52.5
	1974	42.9	103.12		17.8	103.65		21.2	49.3
	1975	42.8	113.50	*	19.1	120.88	***	20.2	50.7
	M	37.1	109.76	*	16.2	113.29	*	21.0	50.0
HS301	1966	40.1	137.79	***	17.6	137.50	***	21.6	50.0
	1969	32.6	142.99	***	14.7	150.00	***	20.3	51.2

Table 3 (cont.)

1	2	3	4	5	6	7	8	9	10
HS301	1970	42.5	128.01	***	19.0	130.13	***	20.2	50.8
	1971	34.6	103.00		16.5	120.43	***	19.8	50.2
	1972	37.2	108.45	*	16.6	114.48	*	20.1	49.2
	1973	36.1	106.80	*	16.6	112.16	*	18.9	52.3
	1974	45.8	110.09	*	18.9	109.88	*	20.7	49.0
	1975	44.7	118.60	***	19.2	121.52	***	19.3	48.8
	M	39.1	115.68	*	17.4	121.68	***	19.9	50.2
HS308	1974	47.2	113.46	*	19.8	115.12	*	21.3	49.6
	1975	41.5	110.37	*	17.5	113.72	*	20.7	47.8
	M	44.3	111.87	*	18.6	112.73	*	21.0	48.7
HS310	1974	48.3	113.70	*	19.9	115.70	*	22.2	49.2
	1975	47.6	126.59	***	19.0	122.81	***	25.5	46.3
	M	47.9	120.96	**	19.4	117.57	**	23.8	47.7

In most hybrids heterosis manifests itself in a decreased percentage of husk and especially greater oil content in seeds. Depending on changes in external conditions, the latter feature is more pronounced in the hybrids than in the Rekord variety, with the exception of the HS310 hybrid.

Summary

1. The three sources of cytoplasmic male sterility obtained in Romania and also the sources of restoring pollen fertility served as the basis for producing many cytoplasmic male sterile lines, fertility restorer lines and the lines resistant to the Plasmopara helianthi. All these forms belong to the most valuable regionalized and experimental sunflower hybrids.

2. The simple hybrids 90, 301, 305, 308 and 310 whose seeds are grown on the basis of cytoplasmic male sterility and restoration of the pollen fertility are resistant to downy mildew and are very powerful, even and fertile.

3. Simple sunflower hybrids have clear advantages over the best ordinary varieties because they make possible a transition to an improved and highly effective system of producing seeds and because they are highly stable, even powerful and resistant to various diseases.