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SINGLE, THREE-WAY AND DOUBLE-CROSSES IN SUNFLOWER

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The impossibility of manual emasculation of sunflower female plants in crossing fields formed for a long time an impediment in obtaining the adequate single crosses. This difficulty was however overcome by developing the male sterile lines.

Although Kuptzov had already called the attention on sunflower sterility in 1935, this was investigated only in the last 15 years and after the particularly valuable results in maize, concerning hybrid seed production based on male sterility were obtained, the investigations progressed tremendously. From then on, different countries have issued papers in which the discovery of various male sterile sunflower types is stressed and the importance of this phenomenon in the production of hybrid seed is emphasized.

The research initiated by us in Romania in the field of sunflower male sterility ended with the description of 3 types of male sterility (Vulpe, 1964 ; 1968) and based on the results obtained with the first single crosses experimented in comparative trials in 1965—1966, we proved the economic importance of sunflower single crosses developed on male sterility basis (Vulpe, 1967).

In the present paper we give the experimental results with sunflower single, three-way and double-crosses.

MATERIAL AND METHODS

Under the favourable conditions for sunflower cropping of 1965—1973 (except 1968 which proved less favourable for single-crosses), different sunflower hybrids, developed on male sterility basis were investigated at the Research Institute for Cereals and Technical Plants.

The hybrid seed was obtained from the male sterile lines genetically marked with anthocyan, by cytoplasmic male sterility and pollen restoration fertility as well as by combining the cytoplasmic male sterile lines with male sterile lines marked with the *T* gene. Table 1 gives the investigated combinations, particularly with respect to segregations in F_1.

Table 1

Combinations and segregations for pollen fertility restoration in F_1, depending on the genetic structure of sunflower hybrid types

Categories of hybrids		Female parent	Male parent	Restored fertility %
Single	1	D[1])	normal	100
	2	B cyt.[2])	restorer	100
cross	3	50% B cyt. + 50% D	restorer	100
	4	50% B cyt. + 50% D	normal	50
	1	D × D mark.	normal	100
Three-way	2	B cyt. × non-restorer	restorer	100
cross	3	50% (B cyt. × non-restorer)+50% (D × D mark.)	restorer	100
	4	50% (B cyt. × non-restorer)+50% (D × D mark.)	normal	50
	1	D × D mark.	B[3]) × normal	100
	2	D × D mark.	V[4]) × normal	100
Double	3	B cyt. × non-restorer	emasculated restorer × restorer	100
cross	4	50% (B cyt. × non-restorer) + 50% (D × D mark.)	B cyt. × restorer	75
	5	50% (B cyt. × non-restorer) + 50% (D × D mark.)	B × restorer	75
	6	50% (B cyt. × non-restorer) + 50% (D × D mark.)	V × restorer	75
	7	50% (B cyt. × non-restorer) + 50% (D × D mark.)	B × normal	50
	8	50% (B cyt. × non-restorer) + 50% (D × D mark.)	V × normal	50

[1]) D monogenic type of male sterility
[2]) B type of cytoplasmic male sterility
[3]) B bigenc type of male sterility
[4]) V polygenic type of male sterility

Experiments on the biological material were carried out using the latin square method with 8 treatments in 4 replications, and variance analysis was used in statistical interpretation.

From 1965 to 1967 the seed and oil yield was expressed for each hybrid in percentage as compared to VNIIMK 8931 grown that time and beginning with 1969 the newly grown variety Record was used as check. Phenological observations and biometric measurements were carried out in the field, while the seed samples taken from each treatment separately in replication plots were analysed in the laboratory as to their physical aspect.

RESULTS

Growth. From the first growth and development stages the three-way and double crosses outdistanced the other treatments by their vigour, but when the heads were formed, there was no striking difference between them and the single crosses. From head formation to the end of anthesis, the three-way and double crosses differentiate from the single crosses regarding uniformity, particularly concerning height, which is in close relation with the parental forms. If the parents of different origin of the three-way and double crosses are alike from every point of view, then the three-way on the double-crosses have the aspect of a uniform and vigorus single-cross.

Therefore, the uniformity in F_1 of a three-way or double-cross hybrid can be anticipated by the ability the breeder has in choosing the parental lines.

Pollen fertility restoration. The cytoplasmic male sterility achieved by us, has allowed us to obtain cytoplasmic male sterile lines from three sources *(H. rigidus x H. annuus,* VNIIMK 8931 and *H. petiolaris x H. annuus)* particularly with the VNIIMK 8931 and *petiolaris* cytoplasm ; they can be used for seed production.

By developing, under greenhouse, 3 generations in one year and even 7 generations in 2 years in the half-early and early plants — we succeeded in transforming the normal inbred lines in cytoplasmic male sterile lines as well as in monogenic male sterile lines by anthocyanic marking. Therefore, the system for producing the hybrid seeds of the two licenced single hybrids HS 90 (Romsun 90) and HS 301 (Romsun 301) is completely new and represents an intermediary way to the exclusive use of cytoplasmic male sterile lines. In each of these two hybrids, the female line is formed by 2 sublines, of which one is genetically marked with anthocyan while the other is based on cytoplasmic male sterility. In crossing fields, where the ratio of the female and male rows is 4 :2, the 2 central female rows are sown with the sub-line genetically marked with anthocyan, while the lateral ones are sown with the cytoplasmic male sterile sub-line. Thus a 50% reduction in manpower is achieved, which would be otherwise strictly necessary for carrying out by stages the different operations necessary for the biological purification of the female line both in multiplication and crossing fields.

Such hybrid seeds produce in F_1 50% male sterile hybrid plants and 50% male fertile hybrid plants which provide a sufficient amount of pollen for the pollination of sunflower fields under optimal conditions. Moreover this proportion of 50% male fertile plants provides an incomparably higher security than a fertility restorer which may, under unexpected conditions, not produce any pollen thus leading to disastrous circumstances.

As a consequence of our previous investigations (Vulpe, 1968), we started and experimented different systems of seed production from single, three-way and double-crosses of sunflower, using numerous combinations among male sterility types, by combining male sterile lines marked with anthocyan with cytoplasmic male sterile lines as well as with normal and fertility restoring inbred lines. Thus, the categories of hybrids that may be developed in the first stage could be experimentally established.

From table 1 it appears that in the category of single hybrids (HS) the presently most convenient treatment is treatment 4, used in our system for developing single-cross seeds of HS 90 and HS 301. For the three-way crosses, such as HTL 1, treatments 1 and 4 are adequate and for double-crosses treatments 1, 2, 7, and 8. The other treatments, of all categories of crosses in which fertility restorers enter, hold great expectations but their application calls for a thorough checking of the restores and for their breeding quality and resistance to diseases.

The growing period (table 2) of all hybrid categories was shorter than in the check variety. Remarkable from this point of view is the three-way-cross 1 (HTL 1) alongside with HS 72 and HS 90.

Plant height of the single, three-way and double-crosses was in general under that of the check, lowest height being recorded for HS 90, HTL 1 and HD 5. The height variability coefficient for the single crosses is very reduced, and this indicates their great uniformity. HTL 1 also proved a great uniformity being more close to the single crosses than to the varieties. Some of the double-crosses were more uniform and others less uniform, depending on the height of their 4 parents. With respect to this they lie between the three-way-crosses and the varieties. Head diameter was in general greater in all hybrid categories as compared to the check and the uniformity was greatest in single crosses, great in three-way crosses and moderately great in double-crosses as compared to the check variety.

1000 kernel weight for the 3 categories of crosses was greater than of the check, while test weight varied, being higher or lower according to the treatment.

Resistance to Plasmopara helianthi. The genetic resistance of sunflower to downy mildew forms one of the main concerns in breeding works. By help of the inbred line S-37-388 RR developed by Putt, a Canadian source resistant simultaneously against downy mildew and rust, many susceptible lines could be changed into lines resistant to downy mildew and rust.

Table 2

Characteristics of different sunflower hybrids

Cultivars	Years of trial	Plant height		Head diameter		Thousand seed weight (g)	Test weight (kg/hl)	Growing period ± over the check
		cm	s %	cm	s %			
HS 72	1965—1968	143	4.7	19.1	8.4	65.7	45.1	—21
	1969—1973	131	4.2	21.6	8.1	64.8	43.5	—16
HS 90	1966—1968	141	4.5	18.9	8.8	64.7	43.9	—10
	1969—1973	129	4.1	22.0	7.8	67.2	41.2	—12
HS 301	1966	158	5.0	21.5	7.9	64.8	42.0	— 3
	1969—1973	157	4.7	21.8	8.2	68.8	40.8	— 7
HTL 1	1973	122	5.6	21.3	9.7	66.2	44.0	—12
HD 2	1973	137	7.8	19.9	10.6	65.4	43.5	— 5
HD 5	1973	125	6.4	20.1	10.3	64.0	43.9	— 6
VNIIMK 8931	1965—1968	194	8.1	18.8	11.3	63.8	41.9	125
RECORD	1969—1973	201	9.8	19.2	11.9	64.0	43.5	128

In our research work, the gene R_1 was transferred to a range of inbred lines, among which the male parent of HS 90, HS 301 and HTL 1 hybrids, as well as to one of the parents of the male fertile single hybrid in the HD 5 formula.

The checking of the biological material with respect to downy mildew resistance was carried out following a rigurous method : it stared with the planting of germinated and infected seeds, in a soil strongly infested with oospores and their spraying right after emergence with a suspension of zoospores.

Although generally sunflower hybrids proved resistant to downy mildew in the field, the scientific test clearly emphasised the difference between the treatments, concerning resistance and susceptibility to this disease, and proved at the same time the efficiency of the artificial infections.

Bearing in mind that the resistance to this disease is monogenically determined and dominant, it is sufficient that only the male parent be resistant in the single or three-way-crosses so as to obtain in F_1 a resistant hybrid. In the case of double-crosses, if a line is resistant to downy mildew, then the double-cross has in F_1 a 50% resistance. It is

Table 3

Productivity of different sunflower hybrids

Cultivars	Years of trial	Yield of seeds q/ha	Yield of seeds %	Yield of seeds significance	Yield of oil q/ha	Yield of oil %	Yield of oil significance	Husks %	Oil in sees %
HS 73	1965	36.6	104.27		15.8	103.95		25.0	49.0
	1966	34.1	117.19	**	14.5	113.28	*	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	48.6
	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	118.24	**	20.1	49.9
	1972	40.6	118.36	**	16.3	112.42	*	20.1	49.2
	1973	35.0	103.55		16.0	108.11	*	20.2	52.2
	M	35.2	111.74	*	15.2	111.76	*	21.1	49.8
HS 90	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
	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
	M	34.8	110.47	*	15.3	112 50	*	21.1	50.0
HS 301	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
	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
	M	36.6	116.18	**	16.7	122.79	**	19.9	50.7
HTL 1	1973	36.8	108.87	*	16.8	113.51	*	20.6	50.8
HD 2	1973	36.2	107.10		16.9	114.12	*	20.5	50.9
HD 5	1973	36.9	109.17	*	16.5	111.49	*	21.1	50.4
VNIIMK 8931	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	1969	22.8	100.00		9.8	100.00		23.8	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
	M	31.5	100.00		13.6	100.00		22.9	48.8

therefore necessary for both the parents of one of the two single-crosses to be homozygous with respect to downy mildew resistance.

Productivity. From the experimental data regarding the seed and oil production in the carried out treatments (table 3), it appears that the sunflower hybrids are superior to varieties. However, when analysing the yields of HS 72 — the first single hybrid developed by us and experimented from 1965 to 1973, — as well as those of HS 90 (1966—1973), we reach the conclusion that seed and oil yield in sunflower single-crosses varies from one year to the other as compared to the check, depending on the weather conditions, and a high heterosis can be achieved only by applying an adequate crop management on irrigated soils.

The problem of single-cross plasticity arises therefore and it can be solved only by replacing them with double-crosses.

Bearing in mind that the first sunflower double-crosses developed by us gave good results, similar to those of single-crosses and even better, we are sure that in a short time such much more economic hybrids will be planted on important areas.

CONCLUSIONS

1. Based on the discovery of 3 male sterility types, on the use of monogenic and cytoplasmic male sterility, and by speeding up the breeding works by developing under greenhouse, 3 generations in one year and for half-early and early plants 7 generations in two years, all possible types of sunflower hybrids (single, thre-way and double-crosses) could be developed in a very short time.

2. Among the single crosses with which trials were carried out for 8 and 9 years, frontranking were HS 90 and HS 301, licenced in 1974.

3. Among the three-way crosses HTL 1 had the best results.

4. Double-crosses HD 5 and HD 2 proved valuable by their hybrid vigor and uniformity being closer to single hybrids than to varieties, as well as by their very high yields.

5. The system of hybrid seed production is based in all hybrid categories on the combination of cytoplasmic male sterility and monogenic male sterility in an 1 : 1 ratio.

6. From an economic viewpoint as well as from that of ecological plasticity, the single hybrids are best suited for irrigated crops, while the double hybrids, followed by the thre-way hybrids are most adequate for non-irrigated crops.

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