Alex. Viorel Vrânceanu,
Florin M. Stoenescu,
Romania

BREEDING FOR POLLEN FERTILITY RESTORATION IN SUNFLOWERS

Obtention of sunflower hybrids on the basis of cytoplasmic male sterility (the Petiolaris type) is most seriously handicapped by an extremely low concentration of genes for pollen fertility restoration in cultivated sunflower (A. Vrânceanu, F. Stoenescu, 1971). The Rf genes are more common in some wild species or in the genotypes (H. Enns et al. 1970; 1972; M. Kinman, 1970; P. Leclercq, 1971; G. Fick et al., 1974).

A voluminous biological material has been investigated at the Research Institute for Cereals and Industrial Crops of Fundulea, allowing the identification of the first cultivated types of sunflowers containing genetic factors for pollen fertility restoration (A. Vrânceanu, F. Stoenescu, 1973). Restorer genes were also identified afterwards in the common cultivars by other authors (D. Scorić, 1974; E. Fernandez, et al, 1974; V. Velkov, Y. Stoyanova, 1974).

I. Sources for Pollen Fertility Restoration

The germoplasm collection existing at Fundulea comprises numerous sources for pollen fertility restoration in progenies with Petiolaris sterile cytoplasm, the majority of them being identified within the domestic breeding material, the others originating from Canada, the USA, France and Argentina.

Each of the eight sources for pollen fertility restoration has one dominant gene responsible for this character.

The Romanian lines MZ-1398, DV-10275 and SL-71-232 have proved to be very good restorers both under field and greenhouse conditions.

They originate from obsolete sunflower cultivars with low oil content and coarse and striped husks. Our statistical results show that the major Rf genes are present in greater quantities in such cultivars than within the present high oil varieties.

The line T-66006-2-1-B comes from Texas, being selected from a composite which included three sources of rust resistance related to wild annual sunflowers. Its restorer gene was designated by Kinman (1970) with the symbol $\underline{Rf_1}$. This source has a good capacity of restoration, but under insufficient illumination, especially in greenhouses, its quantity of pollen is reduced. The same characteristic is present in the line CF-11-73 RF obtained at Fundulea by selfing within the French hybrid CVH 61-BC 23.

The Canadian sources MO-1338 and MO-1356 (H. Enns, 1972) had only a partial restoration under greenhouse conditions in 1975-1976 winter. These sources gave an increased number of sterile plants in the investigated segregation ratios under field conditions too. The constancy of this phenomenon is assured by the high P values for progeny homogeneity, especially for the source MO-1356.

The American source ND-7227 originates from a cross involving the wild annual sunflower species <u>Helianthus annuus</u> (G. Fick et al., 1974), having a very stable restoration capacity.

There are three cases in which the restoration of pollen fertility seems to be determined by the action of two complementary genes. In the first case (S 11-15-72-3) the restoration source was homozygous for the two <u>Rf</u> genes, and consequently the F_1 generation was entirely fertile. In the second case (ND-280) the ratio 1 is fertile: 1 sterile in F_1, as well as the segregation of the selfed father plant indicates that one of the complementary genes was heterozygous and the other homozygous. In the third case (RF-M-1193-3) the plant utilized in crosses was double heterozygous, the F_1 generation containing 25% fertile and 75% sterile plants and the selfed father plant having the segregation ratio of 9:7.

Restoration provided by the first two lines S 11-15-72-3 and ND-280 was very stable. The restorer line S 11-15-72-3 was obtained at Fundulea by selfpollination and selection within the synthetic population "Synthetic 11" which included several interspecific hybrids H. tuberosus x H. annuus. This synthetic population also contains genes for resistance to downy mildew (Plasmopara helianthi) and white rot (Sclerotinia sclerotiorum).

The ND-280 source is based on the American line RHA-280, isolated from the confectionary variety Sundak (G. Fick, D. Zimmer, 1974).

The restoration capacity of the Argentine line RF-M-1193-3 (9) was affected to a great extent by the greenhouse conditions of the 1975-1976 winter.

In some cases, the restoration of pollen fertility seems to be the result of the interaction of more than two complementary genes. The segregation ratios in some cases suggest the existence of three complementary genes which assure pollen fertility only when they all occur in a dominant state (Rfa-Rfb-Rfc). When a monoheterozygote participates in the cross, the ratios 3 fertile: 1 sterile (for F_2) and 1:1 (for test cross) are obtained.

For a double heterozygote such ratios are 9:7 and 1:3 and for the tripleheterozygote 1:1 and 1:7. Some segregating ratios could not be interpreted, because they differed too much from the expected proportions. Such ratios as well as the high frequency of the partially fertile plants, particularly under unfavourable conditions, also indicate the existence of certain modifiers which are very susceptible to the environmental variations. It is likely that some of the complementary genes play the role of modifiers.

The inbred lines OG-817, BL-15 and BL-17 have been selected at Fundulea by selfpollinating within the ornamental sunflowers existing in germoplasm collections. The inbred line V-2618 originates from the Soviet open pollinated variety VNIIMK 8931.

Data from the table reveal another category of genetic control of pollen fertility restoration, namely the cumulative action of two nonallelic dominant genes. The line MH-5383 originates from an old open pollinated variety-population called Manchurian hybrid. The first generation, entirely fertile, indicates the homogeneity of this line for the Rf genes. The clearcut ratios 9:6:1 and 1:2:1 between fertile, partially fertile and sterile plants obtained in the two segregating generations, both under field and greenhouse conditions, suggest that the partial restoration in this case is determined by a single gene Rf, but the complete restoration is effected only by the dominant alleles of both Rfa-Rfb genes.

In practical breeding work is more difficult to utilize polygenic restorers, which have more than two major Rf genes, due to their lability towards the action of various environmental conditions and because they require more complicated breeding and seed production methods.

The restoration of pollen fertility controlled by polygenic complexes also produces a series of surprises in the process of creation of CMS inbred lines. When such a polygenic complex occurs in the genotype of the fertile analogous line B, the analogous line A is completely sterile in the first generations of backcross, but as soon as its genotype becomes saturated with these polygenes the phenomenon of pollen fertility restoration appears, in most cases as a partial fertility. One should avoid + these lines in the programme of sunflower hybrid development.

II. Genes for Pollen Fertility Restoration

Lines or sources for pollen fertility restoration with defined genetic control were included in a system of diallelic crosses, with a view

Table

Segregation Ratios of Crosses Between One Cytoplasmic Male Sterile Line and the Pollen Fertility Restorer MH-5383 (Fundulea, 1974-1976)

Generation	Testing place and year	Number of progenies	Ratio fertile: partial fertile: sterile plants		P %	
			Observed	Theoretical	for segregating progenies	for progeny homogeneity
1	2	3	4	5	6	7
F_1	Field, 1974-75	4	87:0:0	-	-	-
	Greenhouses, 1974-1975	1	29:1 *:0	-	-	-
Male parent	Field, 1974	2	43:0:0	-	-	-
	Greenhouses, 1974-1975	1	26:2 *:0	-	-	-

Table (continued)

1	2	3	4	5	6	7
F_2	Field, 1975	3	43:34:3	9:6:1	0.5–0.7	0.1–0.3
	Greenhouses, 1975–1976	1	12:12:0	9:6:1	0.1–0.3	–
Sterile x F_1	Field, 1975	2	13:45:16	1:2:1	0.1–0.3	>0.05
	Greenhouses, 1975–1976	1	6:16:4	1:2:1	0.3–0.5	–

* The partially fertile plants have been considered fertile

to testing the allelism of the Rf genes. All variants employed in this study contained sterile Petiolaris cytoplasm. In order to obtain the F_1 generation, the female parents were emasculated manually. The F_1 generation was entirely fertile.

When both parents with monogenic restoration possess the same gene Rf in a homozygous state, the F_2 and testcross generations appear entirely fertile. This is the case with the lines MZ-1398, DV-10275, SL-71-232 and ND-7227. Knowing the fact that the Rf gene of the line ND-7227 comes from a wild species of H. annuus, one can assume that the obsolete open pollinated variety-populations such as Mezőhedeshy, Discovolante and Scovenska-siva owe this gene to the wild annual species of the Helianthus genus.

If the parents with monogenic restoration possess different Rf genes in a homozygous state, the F_2 and testcross generations will segregate 15:1 and 3:1, respectively. In the cross CF-11-73 RF x SL-71-232, the F_1 plants were heterozygous for the gene Rf of the line CF-11-73 and for this reason the obtained ratio was 3:1 in F_2 and 1:1 in testcross. The restoration genes of the Canadian lines MO-1338 and MO-1356 were also affected in these crosses by the greenhouse environment (lack of artificial illumination) during the winter of 1975-1976, producing a significant proportion of partially fertile plants. A similar situation was also found in F_2 of the cross DV-10275 x CF-11-73 RF.

The ratios 29:3 and 5:3 in F_2 and testcross respectively prove that the two complementary genes of the inbred line S-11-15-72-3 are independent of the restorer gene existing in DV-10275, SL-71-232 and ND-7227.

Since the gene of the American line T-66006-2-1 B was already named Rf_1, we propose the symbol Rf_2 for the other gene existing in the genotype of the Romanian lines

MZ-1398, DV-10275, SL-71-232 and ND-7227. The restoration capacity of the Rf_2 gene under field conditions is similar to that of Rf_1 both quantitatively and qualitatively, but the Rf_2 gene is less influenced by the greenhouse conditions.

III. Development of Pollen Fertility Restorer Lines

It is desirable to work with those genes which have a constant pehnotypic expression under different environmental conditions, as for instance the nonallelic genes Rf_1 and Rf_2. For making them evident, these genes are always kept in sterile cytoplasm, so that all plants containing restorer genes will be fertile.

The following breeding methods can be utilized for obtaining pollen fertility restorer lines:

1. Selfpollination and selection within the existing single or three-way hybrids produced on the basis of cytoplasmic male sterility with pollen fertility restoration.

2. Selfpollination and selection within synthetic populations containing genes for pollen fertility restoration in sterile cytoplasm.

3. Transference of the Rf genes into the best inbred lines. Initially, a source containing the Rf gene in sterile cytoplasm is used and the heterozygous fertile plants are crossed to the nonrestorer inbred line with artificial emasculation. Only plants containing the dominant allele Rf will be maintained in backcross progenies, the genotypes rf rf being sterile. After 4-6 backcrosses, the fertile plants are selfpollinated, in order to obtain the homozygous restorer analogue in sterile cytoplasm.

If the line which must be converted has a sterile analogue A, any restoration source can be used even if it has no sterile cytoplasm. The sterile analogue A is always employed as a recurrent female parent, avoiding in this way the artificial emasculation, its plants being repeatedly crossed to the heterozygous fertile progenies of each backcross.

Concomitantly with the breeding work for pollen fertility restoration, genes for resistance to diseases, as for instance to downy mildew (Plasmopara helianthi), can be incorporated into the genotype of the male parent, the selection of the fertile resistant plants being done with artificial inoculations in greenhouses and phytotron (A. Vranceanu et al., 1974).

References

1. Vranceanu A.V., Stoenescu F., 1971, Euphytica, 20: 536-541.
2. Enns H. et al., 1970, Proc. Fourth Intern. Sunfl. Conf., Memphis, U.S.A., 162-167.
3. Enns H., 1972, C.R. 5 Conf. Intern. Tournesol, Clermont-Ferrand, France, 213-215.
4. Kinman M.L., 1970, Proc. Fourth Intern. Sunfl. Conf., Memphis, U.S.A., 181-183.
5. Leclercq P., 1971, Ann. Amel. Pl., 21, 1.
6. Fick G.N. et al., 1974, Proc. Sixth Intern. Sunfl. Conf., Bucharest, Romania, 333-339.
7. Vranceanu A.V., Stoenescu F., 1973, Analele I.C.C.P.T. - Fundulea 39 C, 253-260.
8. Scoric D., 1974, Proc. Sixth Intern. Sunfl. Conf., Bucharest, Romania, 405-411.
9. Fernandez E.J. et al., 1974, Proc. Sixth Intern. Sunfl. Conf., Bucharest, Romania, 339-342.
10. Velkov V., Stoyanova Y., 1974, Proc. Sixth Intern. Sunfl. Conf., Bucharest, Romania, 361-366.
11. Fick G.N., Zimmer D.E., 1974, Crop Sci., 14: 603-604.
12. Vranceanu A.V. et al., 1974, Proc. Sixth Intern. Sunfl. Conf., Bucharest, Romania, 367-371.