

## DEVELOPMENT OF FERTILITY RESTORER LINES ORIGINATING FROM INTESPECIFIC HYBRIDS OF GENUS *Helianthus*

---

M. Christov, P. Shindrova, V. Encheva, V. Venkov, L. Nikolova,  
Al. Piskov, P. Petrov and V. Nikolova

---

*Institute for Wheat and Sunflower "Dobroudja", 9520 General Toshevo,  
Bulgaria*

*Received: March 03.1996.*

*Accepted: July 19.1996.*

### SUMMARY

Hybrid material obtained from reciprocal hybridization between the cultivated sunflower and 29 wild *Helianthus* species was used for the production of new sunflower fertility restorer lines. Single and double backcrossing, sib-pollination and self-pollination were applied. New R-lines were developed from the hybrid material, obtained by repeated individual selection and self-pollination, and combining useful agronomic traits from the wild *Helianthus* species and cultivated sunflower - Rf-genes, coming from different species, high seed oil content, resistance to some diseases and broomrape and very good combining ability. Good hybrid varieties with commercial value were produced with the participation of these new R-lines.

**Key words: Sunflower, wild *Helianthus* species, R-lines, agronomic traits.**

### INTRODUCTION

The heterosis of sunflower has been practically applied after discovery of the cytoplasmic male sterility - restoration of fertility genetic system.

The first stable source of cytoplasmic male sterility (CMS) was discovered by Leclercq (1969). Fertility restoration of the first CMS was identified by Kinman in 1970 (Fick et al., 1974). Enns et al. (1970) reported that fertility restorers were present in wild and cultivated sunflower. Leclercq (1971) found a restorer gene in germplasm derived from *H.petiolaris*.

Sources for fertility restorer genes (Rf genes) for the same CMS source were also discovered by Vranceanu and Stoenescu (1971, 1976, 1978), Fick et al. (1974), Skoric et al. (1978), Skoric et al. (1987), Christov and Petrov (1988), Christov and Nenov (1988), Christov (1990), etc.

Wolf and Miller (1985), Serieys (1986), Serieys and Vincourt (1987) have reported about the identification of Rf genes for newly developed CMS sources.

The aim of this study was to present some results of the work at the Institute for Wheat and Sunflower, Bulgaria, on the development of fertility restorer lines (R-lines) using interspecific hybridization in the genus *Helianthus*.

## MATERIALS AND METHODS

The hybrid material was developed by using two-way hybridization between the cultivated sunflower and 29 wild *Helianthus* species. The following accessions of *Helianthus* species were used: *H. annuus* E-004, E-039, *H. argophyllus* E-006, E-007, E-008, *H. debilis* E-010, E-011, E-014, *H. neglectus* E-017, *H. petiolaris* E-023, E-024, E-025, E-034, E-036, E-037, *H. praecox* E-027, E-028, E-029, *H. divaricatus* M-043, M-015, *H. giganteus* M-011, M-030, *H. glaucophyllus* M-012, *H. grosseserratus* M-014, M-031, M-049, *H. maximiliani* M-017, M-018, M-078, M-081, *H. mollis* M-020, M-082, *H. microcephallus* M-080, *H. nuttallii* M-021, M-088, *H. occidentalis* M-023, M-065, *H. salicifolius* M-045, M-078, *H. silphoides* M-025, *H. smithii* M-008, *H. decapetalus* M-043, *H. hirsutus* M-007, M-029, *H. laevigatus* M-016, *H. scaberimus* M-042, M-045, *H. tomentosus* M-041, *H. ciliaris* M-092, *H. eggertii* M-001, *H. pauciflorus* M-002, M-028, *H. resinosus* M-046, *H. strumosus* M-056, M-059, *H. tuberosus* M-004, M-037, M-039, M-050, *H. x laetiflorus* M-005. The variety Peredovik and inbred lines 2607 A, 1607 A, 1234 A, NS 26 A and HA 89A were used for the production of hybrid material. The scheme for developing R-lines was proposed in 1988 by Christov and Petrov. The inbred lines 147 R and HA 89 were used as checks.

The fertile  $F_1$  plants obtained after crossing cytoplasmic male sterile analogues of the lines 3004, 1607, 1721 and HA-89 and the wild species of *Helianthus* were self-pollinated individually under isolation bags. Backcrosses were made with the same sterile forms pollinated by pollen of  $F_1$  hybrid plants. Double backcrosses were made with part of the hybrids and especially with those hybrids obtained by crosses of tetraploid and hexaploid perennial species *H. decapetalus* M-043, *H. hirsutus* M-007 and M-029, *H. laevigatus* M-016, *H. eggertii* M-001, *H. resinosus* M-046, *H. pauciflorus* M-002 and M-028, *H. strumosus* M-056 and M-059, *H. tuberosus* M-004, M-037 and M-039 and *H. x laetiflorus* M-005.

Self-pollination and repeated individual selection were maintained until plant characters were stabilized. The development of R-lines from wild species and the cultivated sunflower started by crossing the male sterile form of sunflower and pollen of  $F_1$ ,  $F_2$  or  $BC_1$  plants from these crosses.

When the objective is to obtain male fertile hybrids, it is necessary to use the method cited above. The  $F_1$  plants derived by crossing wild x cultivated sunflower, which indicated the presence of Rf genes, are also the source for directly obtaining R-lines. An evaluation of oil content and fatty acids composition, resistance to downy mildew, *Phoma*, *Phomopsis*, white rot and broomrape, and biomorphological traits were made on all advanced R-lines. The oil content

measurements were made on dry seeds of self-pollinated material, by nuclear-magnetic resonance. The disease evaluations were made both under field and laboratory conditions. The methods used for evaluation of the material were those used for the wild *Helianthus* species (Christov et al., 1992). The lines 1607 A, 2607 A, 3004 A and HA-89 A were used as testers for evaluation of general combining ability (GCA). The lines which showed good general combining ability were included in hybridization with other lines as 1234 A, 6041 A, 6044 A, 6054 A, 6057 A, 6065 A and 6075 A. The hybrid combinations were tested at IWS Dobroudja.

## RESULTS AND DISCUSSION

The agronomic characteristics of the new R-lines, obtained by interspecific hybridization, showed that there exists great variability regarding R-lines. Short height, branched, and unbranched R-lines were obtained using *H.praecox*, *H.debilis*, *H.annuus*, *H.glaucophyllus*, *H.tuberosus* and *H.x laetiflorus*.

By using *H.argophyllus*, *H.laevigatus* and *H.smithii*, R-lines with taller height were obtained. Many of the branched R-lines had a prolonged flowering stage. Most of the R-lines had early to medium maturity. The head diameter was 11 cm to 26 cm.

Seed size and weight were different with most of the branched R-lines having small to medium volume weight seed. Unbranched plants predominately had medium volume weight of seed. In both of these two types of R-lines, volume weight of seed depended on the direction of selection and parents. The husk color was usually black or grey-black. The 1000 seed weight ranged from 35 up to 82.6 g. Oil content varied from 37.0 up to 52.0%. The R-lines with high linoleic type of oil predominated. Partial characterization of some advanced R-lines and fixed R-lines is given in Table 1 and 2.

Phytopathological evaluation showed that 90 to 95% of the advanced R-lines were resistant to downy mildew. The resistance to *Plasmopara helianthi* along with the Rf genes were transferred from the accessions of the species *H.annuus*, *H.petiolaris*, *H.debilis*, *H.praecox*, *H.tuberosus*, *H.eggertii*, *H.pauciflorus*, *H.x laetiflorus*, *H.ciliaris*, *H.decapetalus*, *H.laevigatus*, *H.scaberimus*, *H.smithii*, *H.glaucophyllus*, *H.giganteus*, *H.grosseserratus*, *H.mollis*, *H.nuttallii*, *H.salicifolius*, etc.

It is difficult to draw a conclusion regarding *Sclerotinia* resistance, but 30 up to 60% of some advanced R-lines originating from *H.annuus* E-092 and *H.annuus* E-007 overcame the attacks of stalk rot after artificial inoculation.

Restoration lines originating from *H.argophyllus*, *H.praecox*, *H.eggertii* and *H.tuberosus* were tolerant to *Phomopsis*.

Restoring lines obtained from some plants of *H.tuberosus*, *H.decapetalus*, *H.debilis* and *H.argophyllus* possessed resistance to broomrape.

Table 1: Characteristics of advanced R-lines developed from wild *Helianthus* species, 1994

Line	Height (cm)	Head diameter (cm)	Days to maturity	Oil content (%)	Generation year	Origin of Rf genes
1319	145	18	102	44.21	11*	<i>H. annuus</i> E-004
1325	120	11	102	40.94	9*	<i>H. debilis</i> E-011
1444	145	13	100	46.52	11*	<i>H. annuus</i> E-039
185	115	13	102	37.39	8*	<i>H. praecox</i> E-028
193	140	15	102	38.33	8**	<i>H. argophyllus</i> E-006
1636	80	11	96	43.00	9*	<i>H. tuberosus</i> M-037
1689	145	11	109	49.52	6*	<i>H. eggertii</i> M-001
1692	130	12	102	44.55	8*	<i>H. pauciflorus</i> M-002
1695	140	13	100	45.83	10*	<i>H. pauciflorus</i> M-028
1718	135	24	108	50.35	9**	<i>H. tuberosus</i> M-004
1743	125	18	100	47.39	11*	<i>H. x laetiflorus</i> M-005
1764	145	22	106	47.09	6**	<i>H. ciliaris</i> M-092
1768	135	18	103	40.79	6*	<i>H. ciliaris</i> M-092
1781	145	19	100	41.24	10**	<i>H. scaberimus</i> M-054
220	160	14	102	43.10	9*	<i>H. smithii</i> M-008
1786	135	15	101	40.25	9*	<i>H. smithii</i> M-008
1796	145	20	100	44.30	9**	<i>H. giganteus</i> M-030
1807	130	18	105	43.08	10*	<i>H. glaucophyllus</i> M-012
223	150	18	95	39.63	10**	<i>H. glaucophyllus</i> M-012
1830	130	16	103	37.27	6*	<i>H. maximiliani</i> M-081
1833	130	21	102	43.18	8**	<i>H. mollis</i> M-020
1860	135	16	103	42.22	9*	<i>H. divaricatus</i> M-044
St.	135	17	108	46.30	n*	147 R
St.	110	22	116	49.82	n**	HA 89

Legend: \* - branched lines; \*\* - unbranched lines

Predominating part of morphological and other traits of the new R-lines, such as plant height, leaf, inflorescence and seed size and shape, 1000 seed weight and sometimes the oil content and vegetation period stand closer to those of the cultivated sunflower. A conclusion could be drawn that the sunflower varieties and inbred lines, participating as parents in the crosses, had significant influence on the phenotype and other plant traits of the new R-lines.

Among the fixed R-lines, line 7009 R showed resistance to *Orobanche* and line 7004 R - resistance to *Phomopsis*. Very good general and specific combining ability were shown by the lines 418/3 R, 7004 R, 7006 R, 7009 R, 7011 R, 7015

Table 2: Characteristics of R-lines developed from interspecific hybrids

Line	Origin of Rf genes	Height (cm)	Head diameter (cm)	Days to maturity	Oil content (%)	Generation (year)
418/3	<i>H. annuus</i> E-003	137	14	110	44.17	10*
7004 R	<i>H. praecox</i> E-028	145	16	112	52.64	12*
7006 R	<i>H. praecox</i> E-028	120	18	98	46.71	12**
7009 R	<i>H. tuberosus</i> M-037	80	13	92	45.99	12*
7011 R	<i>H. annuus</i> E-004	145	15	103	46.85	11*
7015 R	<i>H. debilis</i> E-011	120	15	102	52.10	12*
7018 R	<i>H. praecox</i> E-028	145	16	110	52.10	12*
7024 R	<i>H. tuberosus</i> M-004	140	25	105	48.95	11*
7026 R	<i>H. smithii</i> M-008	140	14	106	45.34	11**
7027 R	<i>H. x laetiflorus</i> M-005	135	17	102	48.86	11*
St.	147 R	135	17	102	46.30	n*

Legend: \* - branched lines; \*\* - unbranched lines

R, 7018 R, 7024 R, 7026 R and 7027 R. The highest percentage of seed set during self-pollination was obtained by the lines 418/3 R, 7004 R, 7006 R, 7015 R, 7024 R, 7026 R and 7027 R. The highest seed yield percentage per sterile head after pollination was obtained by the lines 418/3 R, 7004 R, 7006 R, 7011 R, 7015 R, 7024 R and 7027 R.

The hybrids MX 93 99 and MX 93 299 originating from the line 7009 R possessed immunity to broomrape. The hybrids MX 93 24, MX 93 34, MX 93 94, MX 93 124, MX 93 164, MX 93 184 developed by crossing the line 7004 R had various levels of resistance to *Phomopsis*.

Table 3: Characteristics of some hybrids developed by crossing the lines 7004 R and 7009 R

Hybrid	Seed yield		Oil content %	Oil yield		Height cm	Days to maturity
	kg / ha	% of Standard Average		kg / ha	% of Standard Average		
MX 9394**	2700	101.5	50.9	1374	117.8	168	114
MX 93124**	2720	95.8	47.6	1295	100.9	156	115
MX 93184**	2710	94.8	45.3	1228	98.6	161	115
MX 93244**	2700	94.4	46.6	1258	101.1	163	115
MX 93299*	3100	105.1	47.1	1460	110.4	125	110

Legend: \* - resistant to *Phomopsis helianthi*; \*\* - resistant to *Orobanche cumana*.

Standard - averaged means of hybrids Albena and Super Start

Some of the hybrids, resistant to *Orobanche* and *Phomopsis*, obtained by crossing lines 7004 R and 7009 R are better characterized in Table 3.

All hybrids originating from the lines 7006 R, 7009 R, 7015 R, 7018 R, 7024 R and 7027 R can be distinguished by high oil content and the hybrids originating from line 7015 R showed the highest seed yield.

## CONCLUSION

Restorer genes along with other useful characters were transferred via interspecific hybridization from wild species to the cultivated sunflower. These new restorer lines were included in heterosis selection and had potential to be used for the development of new hybrid combinations of great economic importance.

## REFERENCES

- Christov, M., P. Petrov, 1988. New source of Rf genes for CMS on the basis of *H. petiolaris*. Genetics and Selection, Vol. 21, Nr. 5, Sofia.
- Christov, M., N. Nenov, 1988. Some studies of interspecific hybrids - carriers of Rf genes for CMS of *H. petiolaris* type. Inter. Youth Conf. of Genetics, p.p. 354 - 360, Albena.
- Christov, M., 1990. Study on wild species *Helianthus* with aim of using in sunflower breeding. Dissertation Ph. D., Bulgarian Academy of Agriculture, Sofia.
- Christov, M., P. Shindrova & V. Encheva, 1992. Phytopathological characterization of wild species in the genus *Helianthus* in view of their use in breeding for resistance. Genet. Breed., Vol. 25, p.p. 45-51.
- Enns, H., D. G. Dorrell, J. A. Hoes and W. O. Chubo, 1970. Sunflower research, a progress report. In Proc. of the 4th Int. Sunflower Conf., Memphis, Tennessee, USA, p.p. 162-167.
- Fick, G. N., D. E. Zimmer, J. Domingues-Gimenez & D. A. Rehder, 1974. Fertility restoration and variability for plant and seed characteristics in wild sunflower. In Proc. of the 6th Int. Sunflower Conf., Bucharest, p.p. 333-338.
- Leclercq, P., 1969. Une sterile male cytoplasmique chez le tournesol. Ann. Amelior. Plant., 19, p.p. 99-106.
- Leclercq, P., 1971. La sterile male cytoplasmique du tournesol. I. Premieres studes sur la restauration de la fertile. Ann. Amelior. Plant., 21, p.p. 45-54.
- Serieys, H., 1986. Characterization of some cytoplasmic androsterility sources from *Helianthus* genus. FAO meeting, July 21-25, Sindos, Greece.
- Serieys, H. and P. Vincourt, 1987. Characterization of some new cytoplasmic male sterility sources from *Helianthus* genus. Helia, Vol. 10, p.p. 9-13.
- Skoric, D., L. Cuk, M. Mihaljevic and R. Marinkovic, 1978. New sources of fertility restoration (Rf genes) and downy mildew resistance (Pl genes) in sunflowers. In Proc. of the 8th Int. Sunflower Conf., Minneapolis, USA, p.p. 423-426.
- Skoric, D., J. Atlagic, R. Marinkovic, L. Vasilevic, M. Mihaljevic and S. Masirevic, 1987. Progress report. 1984-1986. FAO Subnetwork, Szeged, Hungary.
- Vranceanu, A. and F. Stoenescu, 1971. Pollen restorer genes from cultivated sunflower (*H. annuus* L.). Euphytica, Vol. 20, Nr. 4, p.p. 536-541.
- Vranceanu, A. and F. Stoenescu, 1976. Breeding for pollen fertility restoration in sunflowers. In Proc. of the 7th Int. Sunflower Conf., Krasnodar, Russia.
- Vranceanu, A. and F. Stoenescu, 1978. Genes for pollen fertility restoration in sunflowers. Euphytica, Vol. 27, Nr. 2, p.p. 617-627.
- Wolf, S. L. and J. E. Miller, 1985. Fertility restoration response of various sunflower cytoplasms. In Proc. of the 11th Int. Sunflower Conf., Mar del Plata, Argentina, p.p. 549-552.

## **CREACION DE LINEAS RESTAURADORAS DE LA FERTILIDAD OBTENIDAS A PARTIR DE CAUCES INTERESPECIFICOS DEL GENERO *Helianthus***

### RESUMEN

El material híbrido obtenido a partir de hibridación recíproca entre girasol cultivado y 29 especies silvestres fue usado para la producción de nuevas líneas restauradoras de la fertilidad. Retrocruzamientos simple y doble, cruces entre hermanos y autofecundación fueron aplicados. Las nuevas líneas restauradoras fueron creadas a partir del material híbrido, obtenidas por selección individual repetida y autofecundación y por combinación de caracteres agronómicos deseables presentes en las especies de *Helianthus* y girasol cultivado: Genes Rf procedentes de las especies silvestres, alto contenido de aceite, resistencia a algunas enfermedades y jopo y muy buena aptitud combinatoria. Se obtuvieron buenos cultivares híbridos con valor comercial con la participación de estas nuevas líneas (R) restauradoras.

## **CRÉATION DE LIGNÉES RESTAURATRICES DE FERTILITÉ À PARTIR D'HYBRIDES INTERSPÉCIFIQUES DU GENRE *Helianthus***

### RÉSUMÉ

Du matériel hybride obtenu à partir de croisements réciproques entre le tournesol cultivé et 29 espèces sauvages d'*Helianthus* a été utilisé pour la fabrication de nouvelles lignées restauratrices de fertilité. On a appliqué les techniques de retrocroisement (on ou deux), de pollinisation frère-soeur et d'autofécondation. Les nouvelles lignées R ont été créées à partir du matériel hybride, obtenu après plusieurs cycles de sélection individuelle et d'autofécondation, combinant les caractères agronomiques intéressants des espèces sauvages d'*Helianthus* et de tournesol cultivé: gènes Rf issus de différentes espèces, teneur élevée en huile, résistance aux maladies et à l'orobanche ainsi qu'une bonne aptitude à la combinaison. Des variétés hybrides à bonne valeur commerciale ont été produites avec la contribution de ces nouvelles lignées R.