FERTILITY RESTORATION ANALYSIS AND GENETIC DETERMINISM OF F2 HYBRID PROGENIES OF THREE CMS SOURCES IN SUNFLOWER

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Abstract
Fertility restoration analysis was tested by crossing 18 inbred lines (as male) with 3 cms sources:ANN3 (from H.annuus sbsp. annuus, female background Ha89), PET2 (from H.petiolaris, female background RHA 265), MAX1 (from H.maximiliani, female background RHA 265). PET2 and MAX1 were the best restored sources whereas ANN3 was restored only by RCMG1 and RHA 801. F2 analysis revealed different segregation ratios, allowing the hypothesis of one single dominant gene, or two complementary genes (9:7), or two non additive homologous genes (15:1).

Introduction

The importance of genetic-cytoplasmic male sterility already discovered in many plant species (Edwarson, 1970) is widely known. In sunflower, the first source of sterility was discovered in H. petiolaris (Leclercq 1969); subsequently many other alloplasmic sources were discovered from wild species.(see Table 1)
Today cytoplasmic male sterility represents the most effective route to convenient economic hybrid seed production. To date, all cultivated sunflower hybrids have the same type of cytoplasm, coded PET1, and the narrow cytoplasmic base may be potentially dangerous for stability and biotic/abiotic stress susceptibility, as much as Maize T cytoplasm for Helminthosporium turcicum (Tatum 1971; Smith and Hooker 1973; Duvick and Noble 1978; Thompson and Berquist 1982). Very important is utilization of, previously unused, newly discovered cms sources (Table 1). All authors on the list tested stability of these sources over a three-year period. Others (Baldini et al.,1991) tested stability of three cms sources (PET2, ANN3, MAX1) over a three year period in two different environments, revealing ANN3 as the stablest source of the three examined. Moreover all these sources lack Rf restorer genes. These genes were discovered in PET1 (Kinman 1970; Enns et al., 1970; Leclercq 1971;) and led to the creation of two restorer lines (RHA 265 and RHA 266) whose fertility was controlled by a (Rr) single couple of alleles. Monogenic inheritance was confirmed by other studies (Vranceanu and Stoenescu 1971) and a 9:7 ratio (complementary and independent genes)(Duvick and Noble 1978) was discovered. Other studies (Dominguez Gimenez and Fick, 1975) identified additional sources of Rf genes, coming from H. annuus and H. petiolaris and a new model explaining F2 segrega-

tion ratio, by the existence of a minimum of 4 loci for this character. F2 Segregation 3:1, 15:1, 63:1 and 255:1 could perhaps be explained. Fertility was restored when at least 2 dominant alleles were present at any of the four loci. The 3:1 ratio may be explained assuming two Rf genes from the male sterile wild population. This model was confirmed by other researchers (Vranceanu and Stoenescu 1976; Vranceanu and Stoenescu 1978; Skoric et al., 1978; Dominguez et al., 1980) and polygenic control was hypothesized, linked to the cumulative action of many dominant genes leading to the breaking of male sterility at BC5-BC6 generations (Vranceanu and Stoenescu 1978). Finally, modifier and environment sensitive genes may be involved in the partial or complete expression of the character. It can be argued that the substitution of PET1 with some other new source is very important. A few authors (Baldini et al., 1991) evaluated the stability expression of 3 male sterility sources (ANN3, Serieys 1987; PET2, Whelan 1980; MAX1, Whelan and Dorrel 1980) in two places and two years. Our paper deals with now the search for fertility restoration lines for three cms sources and the F2, BC1 (in a few cases) segregation ratio.

Materials and Methods

Intraspecific hybridizations were made during 1988 at the Experimental farm of the Agronomy Department, Pisa university, situated at Torretta village (San Piero a Grado, Pisa). Three cms sources (ANN3, PET2, MAX1) at BC6 level (female background HA89, RHA265 and RHA 265 respectively) were crossed with male fertile lines listed in Table 2. Each cross was repeated on ten plants and only half of each female head was pollinated. Pollen was gathered from ten plants and bulked.
During 1989 the following characters were checked:

- pollen shed (pollen yield with a scale 0 = no pollen and 5 = high pollen yield)

- pollen stainability by acetocarmine test (Darlington and La Cour, 1962)(Baldini et al., 1991); ten flowers/slide were scored with three slides for each hybrid combination. Scoring was done over 100 separable fields of vision containing pollen grains for each slide. A preliminary ANOVA was done to check for absence of significant differences among fields of vision within each cms source;

- flower morphology (presence + or absence - of anthers);

- hybrid achene presence (this last character was checked also in another environment, La Tijereta, Entre Rios land, Argentina).

Data were submitted to analysis of variance and when statistically significant differences were observed resulted, in order to compare the mean values among them, the Least

Significant Differences were calculated for P 0.05-0.01. Finally, after creation of F2 and BC1 generations (created also in Argentina), during 1990 segregation ratios of these progenies were evaluated. All available plants were utilized and transplanted manually at the 4 true leaves stage. Fertility and sterility were checked. Data were submitted to X2 analysis.

Results and Discussion

Pollen stainability (Table 3) shows high levels of viable pollen in PET1 x R classical restorer lines and low levels in HA 99 (9.0 %) and HA 89 (0.0 %). ANN3 cms was restored only by RCMG1 (75 % of red pollen grains) and RHA 801 (75 %) while all the other lines revealed low levels (<9.0 %) of red pollen grains in hybrid combinations. PET2 cms source was restored by RHA 279 (77.5 %), PAH2 (95.0 %, fertile genotype), HA 291 (60.0 %, fertile genotype) and ANT 1B (90.0 %, fertile genotype). MAX1 source was easily restored by RCMG1 (92.5%), RHA 279 (92.5), HA291 (92.5) and ANT1B (90.0)(fertile gnotype)(Table 3). Pollen shed was about 5 for PET1 x R hybrid combinations (0 in the others), whereas 2-3 for ANN3 hybrids (0 in the others); PET2 x PAH2, PET2 x RHA 801 and PET2 x ANT1B showed 4-5 pollen shed whereas other PET2 combinations showed lower levels. Good standards were in MAX1 x RCMG1 (4) and MAX1 x HA 291 (4).If one takes into account morphology of disk flowers in all the hybrids, then PET2 combinations show (with exceptions) male fertile morphology with developed anthers, and no pollen or low white pollen yield. These characters confirm the results of the last research on this source (Baldini et al., 1991).

Achene presence or absence in the combinations examined is listed in Table 4. PET1 is regularly restored by R classical lines, and maintained by classical maintainer lines (Ha89 and Ha99) although in Argentina Ha99 gives fertility to PET1. ANN3 is restored by RHA801 in Pisa and Argentina whereas opposing results are in RCMG1 (+ in Pisa, - in Argentina) and RCMG2, PAH2, RHA266, ANT1B (- in Pisa, + in Argentina). PET2 is restored by RHA801 and ANT1B (in both places) whereas RCMG1, PAH2, RHA266, RHA279 are partially or totally restored only in Pisa. Ha89 seems to restore PET2 only in Argentina. MAX1 is restored by RHA279, RHA801, HA291 in both places, by RMG1, RHA271, RHA274, ANT1B only in Pisa and by RCMG3 in Argentina.

F2 segregation analysis shows a 3:1 ratio in PET1 hybrid combinations, revealing Rr monogenic determinism; ANN3 reveals a 9:7 (2 complementary genes ANN3 x RCMG2) and 15:1 (ANN3 x RHA 801, two non additive genes) ratio; in MAX1 segregations one finds 9:7 F2 ratios, showing a complementary couple of genes (Table 6,7,8,9).

TABLE 1 - CMS SOURCES DISCOVERED TO DATE

N°	Codes		Species of origin	Researcher
	Traditional	FAO		
1	cms F	PET1	H.petiolaris	Leclercq 1969
2	K	ANL1	H.annuus lenticularis	Anaschenko 1974
3	CMG1	PET2	H.petiolaris	Whelan 1980
4	CMG2	GIG1	H.giganteus	Whelan 1981
5	CMG3	MAX1	H.maximiliani	Whelan e Dorrel 1980
6	I	ANL2	H.annuus lent	Heiser 1982
7	FUN1	ANT1	H.annuus texanus	Vranceanu et al,1986
8	PF	PEF1	H.petiolaris fallax	Serieys 1987
9	BOL	BOL1	H.bolanderi	Serieys 1987
10	Ex	EXI1	H.exilis	Serieys 1987
11	397	ANN1	H.annuus californicus	Serieys 1987
12	517	ANN2	H.annuus texanus	Serieys 1987
13	519	ANN3	H.annuus arizonensis	Serieys 1987
14	521	ANN4	H.annuus australicus	Serieys 1987
15	NSANN81	ANN5	H.annuus annuus	Skoric 1986
16	NS ANN2	ANN6	H.annuus annuus	Skoric 1988
17	VULPE	RIG1	H.rigidus	Vulpe 1972
18	NEGLECTUS	NEG1	H.neglectus	Serieys 1987
19	PET/PET	PEP1	H.petiolaris pet	Serieys 1987
20	ANOMALUS	ANO1	H.anomalus	Serieys 1987
21	CANESCENS	NIC1	H.niveus canescens	Serieys 1987
22	ARGOPHYLLUS	ARG1	H.argophyllus	Christov 1990
23	ARGOPHYLLUS	ARG2	H.argophyllus	Christov 1990
24	PRAECOX	PRP1	H.praecox praecox	Serieys 1987

TABLE 2 - LIST OF MALE FERTILE LINES UTILIZED

HA89, HA99 (Leclercq 1969) da Vniimk 8931
HA291 (Fick et al.,1979)
ANT1B (Vranceanu 1986)
RCMG1,RCMG2,RGMG3 (Wolf e Miller 1985; Whelan e Dedio 1980)
PAH2 (Serieys 1987)
RHA265, RHA266 (Kinman 1970)
RHA271 (Fick e Zimmer 1974)
RHA274, RHA276, RHA278, RHA279 (Fick et al., 1975)
RHA298, RHA299 (Fick et al., 1979)
RHA801 (Roath et al., 1981)

TABLE 3 - FERTILITY RESTORATION ANALYSIS IN F1 HYBRIDS
(POLLEN STAINABILITY)
(% RED POLLEN GRAINS)

LINES	CMS SOURCES			
	PET1	ANN3	PET2	MAX1
HA89	0.0n^	0.0n	0.0n	25.0gk
RCMG1	*	75.0cd	0.0n	92.5ac
			90.5ac $$$$$	
RCMG2	92.5ac	7.5kn	0.0n	35.0fh
RCMG3	*	0.0n	0.0n	25.0gk
PAH2	*	7.5kn	95.0ab (f)	30.0gi
			5.01n (s)	
RHA265	96.5a	2.5mn	9.0jn	12.5 in
RHA266	98.5a	0.0n	5.01n	60.0de
RHA271	91.5ac	0.0n	0.0n	50.0ef
RHA274	97.5a	2.5mn	0.0n	30.0gi
RHA276	91.5ac	0.0n	0.0n	27.5gj
RHA278	97.0a	0.0n	0.0n	22.5gl
RHA279	90.5ac	0.0n	77.5bd	92.5ac
RHA298	91.0ac	4.01n	0.0n	20.0hm
RHA299	92.0ac	1.0n	0.0n	17.5hn
RHA801	90.5ac	75.0cd	92.5ac	32.5fh
HA99	9.0jn	0.0n	0.0n	12.5in
HA291	*	9.0jn	60.0de (f)	92.5ac
			2.5mn (s)	
ANT1B	*	7.5kn	90.0ac (f)	90.0ac (f)
			40.0fg (s)	20.0hm (s)

* = not obtained crosses for plant death

(f)=fertile genotype
(s)=sterile genotype
LSD: 18.5 (0.05); 24.6 (0.01)

$$$$$ = a plant was sterile with a big anther in the centre of the head and generated 5 achenes each with three cotiledons

^Values followed by common letters are not significantly different for P 0.05

TABLE - 4 FERTILITY RESTORATION ANALYSIS OF F1 HYBRIDS.
ACHENE YIELD (+ = WITH ACHENES)
(- = WITHOUT ACHENES)

LINES	CMS SOURCES			
	PET1	ANN3	PET2	MAX1
HA89	-	-	-A	-
RCMG1	*	+(a)	+^^^	+(a)
RCMG2	+(a)	-A	-	-
RCMG3	*	-	-	-A
PAH2	*	-A	+$	-
RHA265	+(a)A	-	-	-
RHA266	+A	-A	+^	-
RHA271	+A	-	-	+(a)
RHA274	+(a)A	-	-	+(a)
RHA276	+(a)A	-	-	-
RHA278	+(a)	-	-	-
RHA279	+(a)A	-	+^^	+$$A
RHA298	+	-	-	-
RHA299	+A	-	-	-
RHA801	+(a)A	+(a)A	+(a)A	+(a)A
HA99	-(a)A	-	+	-
HA291	*A	-	-^^^^	+A
ANT1B	*	-A	+A^^	+$$$

* = not obtained crosses for plant death
A = fertility in Argentina;
$ = fertility present in a plot, in 8/19 plants
$$= fertility present in a plot, in 8/15 plants
$$$=fertility present in a plot, in 5/8 plants

^ = few achenes obtaned and in lateral heads
^^= obtained achenes in 2/3 plants
^^^= a plant was sterile with a big anther in the centre of the head and generated 5 achenes each with three cotiledons
^^^^= a sterile plant had a fertile sector in the head

a = crosses repeated in 1990

TABLE 5 - F2 AND BC1 SEGREGATION RATIOS ANALYSIS OF THE HYBRIDS

Hybrids	F : S	X2	P
PI PET2 x ANT1B P2F2	15 : 1	0.028	0.5<P<0.9
PI (PET2xANT1B)xPET2	1 : 3	3.53	0.05<P<0.1
	1 : 7	0.19	0.5<P<0.9
AR PET2xANT1B 1-F2	-----	All sterile plants	
PI PET2 x PAH2 p126	3 : 1	0.02	0.5<P<0.9
PI PET2 x PAH2 P3F2	3 : 1	0.56	0.1<P<0.5
PI PET2 x PAH2 P2F2	3 : 1	0.083	0.5<P<0.9
PI PET2 x PAH2 P1F2	3 : 1	0.249	0.5<P<0.9
PI PET2 x PAH2 P1F2	3 : 1	0.0145	P>0.9
AR PET2 x HA89 1-2F2	1 : 7	0.0166	P>0.9
AR PET2 x HA89	1 : 3	0.0074	P>0.9
	1 : 7	4.823	0.01P<0.05
AR PET2 x RHA801 F2	3 : 1	0.0565	0.5<P<0.9
AR (PET2 x RHA801)xPET2	1 : 7	5.46	0.01<P<0.05
	1 : 3	7.59	0.001<P<0.01
	1 : 1	0.195	0.5<P<0.9
AR (PET2 x RHA801)p85	3 : 1	0.00851	P>0.9
PI PET2 x RHA801 p2F2	9 : 7	0.0454	0.5<P<0.9
AR PET2 x RHA801 F2	1 : 3	0.000001	P>0.9
AR PET2 x RHA801 F2	1 : 3	0.13	0.5<P<0.9

PI=PISA AR=ARGENTINA P = significance level

F=Fertile S=Sterile

TABLE 6- F2 AND BC1 SEGREGATION RATIO ANALYSIS OF THE CONSIDERED HYBRIDS

Hybrids	F : S	X2	P
PI MAX1 x ANT1B F2P4	9 : 7	0.000001	P>0.9
PI MAX1 x ANT1B	9 : 7	0.01022	0.5<P<0.9
PI MAX1 x ANT1B	9 : 7	0.125	0.5<P<0.9
PI MAX1 x ANT1B	9 : 7	0.022	0.5<P<0.9
PI MAX1 x ANT1B	9 : 7	0.119	P>0.9
PI MAX1 x ANT1B	9 : 7	0.160	0.5<P<0.9
PI MAX1 x ANT1B	9 : 7	0.311	0.5<P<0.9
PI MAX1 x RHA279 P2F2	9 : 7	0.000228	P>0.9
PI MAX1 x RHA279	9 : 7	0.6579	0.5<P<0.9
PI MAX1 x RHA279 P1F2	3 : 1	0.225	0.5<P<0.9
AR MAX1 x RHA279 -4F2	9 : 7	0.0039	0.5<P<0.9
AR MAX1 x RHA279 F2	3 : 1	0.00001	P>0.9
PI (MAX1 x RCMG1) x MAX1	3 : 1	1.48	0.1<P<0.5
PI MAX1 x RCMG1 P1F2	9 : 7	0.0189	0.5<P<0.9
PI MAX1 x RCMG1 P2F2	9 : 7	0.0657	0.1<P<0.5
PI MAX1 x HA291 P2F2	9 : 7	0.004	0.5<P<0.9
PI MAX1 x HA291 P1F2	9 : 7	0.00332	0.5<P<0.9
PI MAX1 x HA291 P3F2	9 : 7	0.0914	0.5<P<0.9
AR MAX1 x HA291	3 : 1	0.082	0.5<P<0.9
	9 : 7	0.9	0.1<P<0.5
AR MAX1 x RHA801	9 : 7	0.031	0.5<P<0.9
AR MAX1 x RCMG3	-----	All fertile plants	
AR ANN3 x ANT1B-8 F2	3 : 1	0.00641	0.5<P<0.9
AR ANN3 x ANT1B	-----	All sterile plants	
AR ANN3 x RCMG2-4 F2	9 : 7	0.009	P>0.9
AR ANN3 x RHA266	-----	All fertile plants	
AR ANN3 x PAH2	-----	All sterile plants	
AR ANN3 x RHA801	15 : 1	0.0056	P>0.9

PI=PISA AR=ARGENTINA P=significance level

Conclusion

A general model explaining such data is quite difficult to describe. In fact many of these results are environment dependent, so that the expression either of the nuclear genes involved and of the nucleus-cytoplasm interaction are highly influenced by environmental conditions. Nevertheless the presence of four or five loci of non allelic genes, involved in fertility restoration can be confirmed. In this case ANN3 would bring only one or no factors of restoration and possibly only RHA 801 and ANT1B could possess all these factors and restore all three sources examined.

This research underlines the potential usefulness of utilization of an ANN3 source in plant breeding programmes. Utilization of restorer and maintainer lines could be very important in a near future. The new task for breeders would then consist in transferring these sources, restorer and maintainer lines, into new high quality genetic germplasm.

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