EVALUATION OF SOME MAIZE INBRED LINES ON FERTILITY RESTORATION PATTERNS OF MALE-STERILE CYTOPLASMS

Voichiþa HA[a*)]

ABSTRACT

The aim of this investigation consisted in detecting the presence of dominant alleles of *Rf* genes in more than 600 inbred lines provided from the inbreds collection of the Maize Breeding Laboratory, at the Agricultural Research and Development Station Turda. They were crossed with different types of cytoplasmic male sterility: *cms*-C, *cms*-ES, *cms*-M and *cms*-T. During 1995-2001, at the Agricultural Research and Development Station Turda, the observations of pollen restoration reactions were scored. The fertility restoration data clearly showed the relationship between the two representative C and ES of the group-C. Thirty-four % of the inbreds maintained the male sterility induced by *cms*-C and *cms*-ES, 55% proved to be restorers and 7% gave partially fertile plants. Many genotypes (27%) were imperfectly sterile and 33% were partially fertile plants, in *cms*-M, meaning that for these genotypes, the fertility was easily affected by environment. In the case of restoration reactions of the inbreds in interaction with *cms*-T, only 16% of these, were fully fertile. Generally, the pollen fertility restoration reactions of the inbred lines were in connection with *cms*-source, *cms*-versions, of several inbred backgrounds, nuclear x cytoplasmic interaction and environmental conditions. In order to use the cytoplasmic male sterility of different types, in maize hybrid seed production, it is necessary to continue the research for finalizing the *cms*-analogues, breeding by backcrossing and selection.

Key words: inbreds, cytoplasmic male sterility, pollen fertility restorers.

INTRODUCTION

The utilization of cytoplasmic male sterility (*cms*) in maize hybrid seed production has represented an increasing source of economical efficiency and of improvement of seed genetical purity.

The hybrid seed production on the basis of cytoplasmic male sterility imposes to the breeders the task of transformation of maternal forms into male sterile analogues and the paternal ones into pollen fertility restorers. This transformation could be achieved only having genotypes with well-known reaction versus certain cytoplasmic male sterility types.

The complex feature of male sterility cytoplasms and pollen fertility restorer genes interaction imposes the necessity to identify the alleles *Rfrf* composition at maize inbreds, with a view to their utilization either as maternal genitors or paternal forms (Sarca and Barbu, 1982; Cabulea et al., 1987; Ciobanu and Partas, 1998; Has et al., 2002).

Nowadays, the most utilized male sterility sources for hybrid seed production are the *cms*-C and *cms*-S types. In spite of the resistance advantages of the cytoplasmic sources C and S to the bacterial leaf blight incited by the race T of *Helminthosporium maydis*, certain authors as Duvick and Noble (1978), Gracen et al. (1979) and Wise et al. (1999), have drawn the attention to the danger that in few years, if only the two C and S sources will be utilized, it may be possible to arrive again at the narrowing and vulnerability of the genetic base.

For preventing this risk, Duvick and Noble (1978), Nemeth (1981), Zeng et al. (1999) suggest the utilization in maize hybrid seed production of a „multiplasm" which, by the genetically diversification of cytoplasm, would prevent the unilateral evolution of pest specialized races.

After Josephson et al. (1978), Darrah and Zuber (1986), some USA companies produce hybrid seeds on the basis of cytoplasmic male sterility, of different types, on about 40% from the seed production plot area.

The pollen fertility restoration is ensured by the action and interaction of a set of dominant genes. In order to restore the *cms*-T cytoplasm type, the presence of two dominant genes with Rf_1Rf_2 complementary action as well as the pres-

*) Agricultural Research and Development Station (A.R.D.S.), 401100 Turda, Cluj County, Romania

ence of some modifying genes for achieving a complete fertility, is necessary (Duvick, 1965; Wise et al., 1999).

A single Rf_3 dominant gene is necessary for the *cms*-S cytoplasm restoration. The Rf_3 dominant gene expression is strongly influenced by the environmental conditions, conclusion expressed in the papers of the following authors: Duvick and Noble (1978), Kheyr- Pour et al. (1981), Laughnan and Gabay-Laughnan (1983).

As regards the genetic control of fertility restoration for the C cytoplasm type, several opinions have been advanced. Josephson et al. (1978), Kheyr-Pour et al. (1979), Kheyr-Pour and Gracen (1980), Laughnan and Gabay-Laughnan (1983) sustain that for the *cms*-C fertility restoration type, at least two genes, Rf_4 and Rf_5 genes, would be necessary.

Having in view the practical importance of cytoplasmic male sterility and pollen fertility restoration in maize hybrid seed production and the necessity of cytoplasm sources diversification, the aim of this paper was the identification in inbreds genotypes of the recessive or dominant alleles Rf_1Rf_2 and Rf_4Rf_5 gene complexes as well as Rf_3 gene with a view to their utilization in genetical transformation programme of parental forms at Turda Agricultural Research and Development Station.

MATERIALS AND METHODS

As biological material, more than 600 inbred lines and cytoplasmic male-sterile analogues, types: *cms*-C, *cms*-ES, *cms*-T, *cms*-M of seven lines, from the maize inbred lines collection of Maize Breeding Laboratory as part of Turda Agricultural Research Development Station, were utilized. During 1995–2001, for the identification of the dominant or recessive alleles, of Rf_1Rf_2, Rf_4Rf_5, Rf_3 genes at some inbred lines, the F_1 hybrid progenies, obtained by topcross in single crosses nursery, have been assessed (by degree of pollen-fertility restoration).

As testers, the male sterile-analogues of some inbreds with identified allelic composition, were utilized; they are also utilized as maternal forms of some perspective hybrids. The F_1 hybrid generations were grown in the observation nursery, on plots of one row of 5 m per each hybrid.

According to pollen fertility restoration degree,the inbreds were classified in six groups (after Josephson et al., 1978):

– group I: no anthers extended;

– group II A: less than half of the anthers extended and all were small, dry and hard, without pollen;

– group II B: most of anthers extended but all were small, dry and hard without pollen;

– group III: partially fertile anthers extended with some pollen shed; the proportion of anthers extended was highly variable;

– group IV (fertility restorers): slightly abnormal anthers with 75 to 100% extension;

– group V (fertility restorers): normal anthers, fully fertile.

In the case in which some inbreds manifested an unstable behaviour depending on the environmental conditions or *cms*-tester, their experimentation was repeated during still two-three years.

RESULTS AND DISCUSSION

In the process of cytoplasmic male steri-lity utilization in maize breeding programmes, it is as necessary as difficult to identify the inbred lines by the composition of the *Rf* gene alleles.

The identification of the pollen fertility restorer genes at some inbreds consisted of the determination of their reaction to the crossings with male sterile testers *cms*-C, *cms*-ES, *cms-T, cms*-M (Table 1).

In interaction with the C and ES cytoplasms, the pollen fertility restorers identific ation is more complicated, due to the implication of at least two - three Rf_4, Rf_5, Rf_6 complementary genes and some modification factors, probably quantitative ones, which under certain environmental conditions, would act in the absence of *Rf* gene, influencing the reaction of lines by the appearance of the „*late-break*" phenomenon (Kheyr-Pour et al., 1981).

Due to the presence of these factors, the observations on lines reaction in C and ES cyto-

plasms must be performed in a period of two-three weeks since the emergence of silking. This phenomenon has been recorded in 8% of the inbred lines tested to *cms*-C, respectively 9% in *cms*-ES (Tables 2 and 3).

Partas (1998). The 24 lines which have proved to be restorers (Table 4) contain in their genotype the Rf_3 dominant allele and they are recommended as genitors in crossing systems or as parental forms. The lines which partially restore the fertil-

Table 1 . Distribution of lines according to their reaction to different types of cytoplasmic male sterility: C, ES, M, T.

Cytoplasm	No. of *cms* tester-lines	No. of tested lines	% lines			
			Non-restorers (*rfrf*)	Restorers		With different reaction
				partially (*pRf*)	complete (*Rf*)	
cms-C	1	5	40	0	60	0
	1	27	52	0	48	0
	1	33	21	9	61	9
	1	95	28	5	60	7
	1	38	48	0	39	13
Total	5	198	-	-	-	-
Average	-	-	34	4	55	7
cms-ES	1	22	41	4	55	-
	1	29	17	7	66	10
	1	43	47	6	47	-
Total	3	94	35	5	51	9
cms-M	1	32	16	9	19	56
	1	89	21	42	20	17
Total	2	121	-	-	-	-
Average	-	-	47	33	20	33
cms-T	1	5	60	0	20	20
	1	37	84	3	13	0
	1	4	0	0	0	100
	1	93	85	0	10	5
	1	29	79	0	21	0
	1	39	61	0	21	18
	1	16	37	6	13	44
Total	7	223	-	-	-	-
Average	-	-	83	1	16	24
Total tested lines		636				

The proportion of non-restorer genotypes (rf_4, rf_5, rf_6) was of 41% in *cms*-C, respectively 44% in *cms*-ES. As a result of analysing the test-crossings, 56% and 54% of the lines were indentified for completely restoring pollen fertility, which certifies the presence of Rf_4, Rf_5, homozygous-dominant alleles in their genotypes (Tables 2 and 3). The other lines (4, respectively 6%) with partial restoration, include probably, dominant alleles for at least one *Rf* gene.

A number of 121 lines have been tested with the *cms*-M cytoplasm, out of which only 20% were identified as pollen fertility resto-rers, which agrees with the results published by Ciobanu and

ity or they have a variable reaction depending on the environ- mental conditions, represent 27% of the lines tested on *cms*-M. This instability is also mentioned in the special literature by Duvick and Noble (1978), Kheyr-Pour et al. (1981), Laughnan and Gabay-Laughnan (1983). The lines which partially restore the fertility or have an unstable behaviour, are not recommended as parental forms, in reproducing hybrids with improved formula on the basis of *cms*-M.

Concerning the reaction to *cms*-T, 223 inbreds were tested, of which 74% have proved to be *non-restorer* (rf_1, rf_2). This fact certifies that the tested lines include only a restorer gene,

probably Rf_2 gene, more frequent at original inbreds of the Corn Belt (Duvick, 1966). Because the *cms*-T is only used in areas less favourable to *Helminthosporium maydis* T-race, the researches on the use of this *cms* type are limited (Table 5).

Table 2. Testing of restoration ability of *cms*-C type at some inbred lines at A.R.D.S. Turda, during 1995–2001

Tester-lines *cms*	Non-restorer inbred lines (*rfrf*)		Fertility restorer inbred lines (*Rf*)	
	Group I	Group II A	Group V	Group IV
W633 *cms*-C	K2051, K2057B,		TC109A, TC221, TB364	
TC208 *cms*-C	TC184, TE235, Lv92, Lv94, Lv113, Lv1700, TC317, TC371, TB368, TB363, TC316, A665, TC333, TC382		TD235, TD218, TB369, TC322, TC314, TC335, TC331, TC327, TC328, K1077, K1042, K1080, P131	
TA 367 *cms*-C	TD106, TD221, TE233, TD269, TC344, TD359, TA417	PT9704 ,TD301, TD344	TD232, TD236, TD237, TD241, TE208, TE220, TB346, TC330, TC331, TD330, TD336, TD350, TD351, TD352, TD355, TD356, TD357, TD358, TA436, K3159B,	TD238, A439, TA435
TC243 *cms*-C	TD268, TE201, TE210, TC318, TC365, TC383, TC384, TC385, TC391, TC395, TC399, TD341, TD347, TA425, TA432, TA438, F426, MV463, P951, K18018, K2148C, K2443B, K3056A, NF1042, NF1109, NF2028,	TD298, TE223, TD320, TA419, TA431, K1801B, NF1032	TD287, TD263, TD279, TD289, TD296, TD297, TD299, TE203, TE206, TE213, TE221, TE226, TE227, TE228, TC339, TC357, TC360, TC361, TC362, TC364, TC368, TC373, TC379, TD329, TD335, TD342, TD345, TD353, TD354, TA430, TA431, TA433, TA437, TA438, TA441, F1218, F1188/89, F858/89, K1653, K1795, K1796, K1800B, K1802A, K1805C, K1511, K2188, K2227, K3041A, K3046B, NF1005, NF1041, NF1090, NF1091, NF1092, NF1093, NF1098, NF1111,	TD278, TE202, TD369, NF1103, TD320
TC184 *cms*-C	TD283, TD284, TC396, TC397, TC398, TD302, TD303, TD304, TD305, TD346, TA426, TA427, F43/93, F768/82, F1870/86, PT9712, PT9714, K2174	TD301, TD344, TD348, TA424 PT9704	TD290, TE217, TC386, TD336, TD346, TD359, TA435, F40/88, PT9707, PT9708, PT9716, PT9717, K3043, K3044, NF1014	
Proportion	**34%** (67/198)	**7**%(15/193)	**55**% (108/198)	**4**%(8/198)

Table 3. Testing of restoration ability of *cms*-ES type at some inbred lines at A.R.D.S. Turda, during 1995–2001

Tester-lines *cms*	Non-restorer inbred lines (*rfrf*)		Fertility restorer inbred lines (*Rf*)	
	Group I	Group II A	Group V	Group IV
TC208 *cms*-ES	TC184, T248B, TC243, TD242, TB367, TB368, TC316, TC317, TC371		TC218, T241, TB369, TC314, TC322, TC328, TC335, K1042, K1077, K1080, P131, W401	TC 327
TC243 *cms*-ES	TE201, TC383, TC384, TC385, NF 2028	TD298, NF1032, NF1109	TD278, TD296, TD297, TD298, TD299, TE202, TE203, TE206, TC330, TC378, TD361, NF1005, NF1041, NF1090, NF1091, NF1092, NF1093, NF1098, NF1111	TD369, NF1103
TA367 *cms*-ES	TD106, TE223, TE222, TE225, TE 235,TD341, TD346, TD 348, TA432, TA438, WN7, F426/88, F1153/89, F43/93, Lv 86, Lv 92,	TD320, TD348, TA439, F768/82, PT9714	TC241, TD263, TE208, TE217, TE220, TE221, TE226, TE227, TD329, TD330, TD354, TA431, TA431, F1218, F1188, F40/88,	TE202, PT9716

Table 4. Testing of restoration ability of *cms*-M type at some inbred lines at A.R.D.S. Turda, during 1995–2001

Tester-lines *cms*	Non-restorer inbred lines (*rfrf*)			Fertility restorer inbred lines (*Rf*)	
	Group I	Group II A	Group II B	Group V	Group IV
MKP33 *cms*-M	TC245, TE207, TB329, TB371, K1075	T248, T291, TC245, TB362, TB366, TB371, P17,W153R,W 633, A665, ND245	TC209, TC243, TA367, TA404, K1079, W117, CM105	Precoce46, CM25, CO216, CO255, A661, K2006	T169a, TC208, TC114
TC 208 *cms*-M	TC109B, TC114, TC184, T243, T248, TD221, TB362, TB366, TC321, TC328, TC331, TC335, TC384, TC385, TA419, K1075, P17, PT9716, ND245	T291, TC243, TC245, TD241, TB364, TB371, TC331, TA404, TA432, K3048A, CO216	A661, TC209, K1080, PT9707	T248B, TC242, TD233, TD283, TE210, TB363, TB367, TB369, TC386, TC391, TC395, TC396, TC397, TD303, TA424, TA428, TA436, PT9708	TD106, TC287, TD233, TD284, TD290, TE208, TA367, TC314, TC344, TC379, TC399, TD301, TD302, TD359, TA425, TA426, TA427, TA429, TA433, TA435, K1042, K1077, K1801B, K2051, K3046B, PT9704, PT3905, PT9710, PT9711, PT9712, PT9713, PT9714, PT9715, PT9717, A665, CM25, CO255
Proportion	**20 %** (24/121)	**18 %** (22/121)	**9 %** (11/121)	**20 %** (24/121)	**33 %** (40/121)

Distribution of inbreds in groups on the basis of fertility restoration, performed by Josephson et al. (1978):
Group I – no anthers extended
Group II A – less than 1/2 of anthers extended, but they are small, dry and hard, without pollen.
Group II B – most of anthers extended, but they are small, dry and hard, without pollen.
Group III – partially fertile anthers extended with some pollen shed. Proportion of anthers extended was highly variable.
Group IV – slightly abnormal anthers with 75-100% extension.
Group V – normal and completely fertile anthers.

Table 5. Testing of restoration ability of *cms*-T type at some inbred lines at A.R.D.S. Turda, during 1995–2001

Tester - lines *cms*	Non-restorer inbred lines (*rfrf*)		Fertility restorer inbred lines (*Rf*)	
	Group I	Group II A	Group V	Group IV
W633 *cms*-T	TC184, TC221, K2051	K2057B	TC109A,	
TC208 *cms*-T	TC182, TC246, TD232, TD233, TD235, TD236, TD242, TB365, TB368, TB369, TC313, TC314, TC322, TC327, TC328, TC331, TC333, TC335, TC344, TA416, K1042, K1077, K1080, K2131, P22, P131, CO120, W401, A661, A665		TC317, TB331, TC316, TC360, TC364	TC371
TC243 *cms*-T		**TD345,** TC394, TD359, TD335		
TA367 *cms*-T	TD268, TD276, TD278, TD296, TD297, TD298, TD299, TE201, TE203, TE206, TE233, TC337, TC351, TC361, TC362, TC365, TC368, TC378, TC380, TC381, TC383, TC384, TC385, TD336, TD343, TD344, **TD345,** TD350, TD351, TD352, TD353, TD355, TD356, TD357, TD358, TD359, TD360, TD361, TA419, TA425, TA430, TA433, TA441, P951, MV463, PT9711, PT9712, PT9713, PT9714, PT9715, PT9716, K1093, K1511, K1795, K1796, K1800B, K1802A, K1805C, K1806, K2148C, K2274, K2308, K2443B, K3041, K3161B, NF1005, NF1014, NF1032, NF1041, NF1042, NF1065, NF1090, NF1091, NF1092, NF1098, NF1103, NF1111, NF2028, NF2188	TD279, TE202, TE212, K2274, TC384	TC326, TD359, TE210, TC395, TC391, TC399, TD287, TD289, K1653	
TC 208 *cms*-T	TC287, TD284, TE208, TE223, TC379, TC386, TD301, TA426, TA428, TA429, TA431, TA432, TA435, PT9701, PT9703, PT9704, PT9705, PT9707, PT9708, PT9710, K3043, K3044, K3046A		TC391, TC397, TC399, TD302, TD303, NF2028	
TC335 *cms*-T	TC241, TE217, TE220, TE222, TE225, TE226, TE227, TD320, TD329, TD330, TD341, **TD345,** TD346, TD347, TD348, TD354, WN7, F426/88, F1970/86, F1188/89, F858/89, F1153/89, F768/82, K2174	TE202, TE223, TE221, TE226, TE227, F1870/86, F1970/86	TD263, TD287, TD289, TC360, F1218, TD290, TD287, TD289,	
TC184 *cms*-T	TD342, K1801B, K3056A, K3159B, PT9707, PT9717,	TD221, TE212, TE227, TE230, TE231, PT9706, PT9711	TD335, TE229	TE228,
Proportion	**74 %** (165 / 223)	**9%**(21/223)	**16 %** (35 / 223)	**1%** (2/223)

A special behaviour has been noticed on the inbred lines T 169a and TD 345 (Table 6).

Table 6. Behaviour of some inbreds with different testers – *cms*

Tester-lines *cms*	Non-restorer inbred lines (*rfrf*)	Fertility restorer inbred lines (*Rf*)
S 42 *cms* T	-	T 169a
W 33 *cms* T	T 169a	-
T 153 *cms* T	T 169a	-
T 248 *cms* T	T 169a	-
A 218 *cms* T	-	T 169a
TA 367 *cms* T	TD 345	-
TC 335 *cms* T	TD 345	-
TC 243 *cms* T	-	TD 345

They presented a different reaction of maintenance or restoration of pollen fertility in relation to the maternal genotype. This fact is explained by the presence of Rf_1 gene in the male sterility genotype analogues of lines S 42 *cms*-T, A 218 *cms*-T, TC 243 *cms*-T respectively, which in interaction with the complementary gene Rf_2 (more frequently in the genotype of some inbreds) into the genotype of T 169a, TD 345 inbreds, achieves the complete restoration of *cms*-T type. This more special behaviour of these two inbreds sustains the necessity to verify the inbreds reaction on both many male sterility types and different *cms* genotypes. This conclusion was encouraged by Duvick and Noble (1978) too, who underlined the importance which should be given to the choosing of some *cms* testers with a clear reaction, no matter of nuclear-cytoplasmic interaction or environmental conditions.

CONCLUSIONS

The behaviour of different lines towards male sterility has manifested as a specific feature dependent on the used male sterility source, genotype of *cms*-analogue, specific character of the nuclear-cytoplasmic interaction and environmental conditions.

The frequency of lines with dominant *Rf* genes manifested for the *cms*-C and the *cms*-ES was of 55%, respectively 51%, in comparison with the proportion of only 16% lines, in the genom of which the dominant genes with complementary action Rf_1Rf_2 useful for *cms*-T restauration are present.

The verification of inbreds to *cms*-M(S) type emphasized the high proportion (33%) of lines with an unstable behaviour; therefore these lines should not be recommended as parental forms for improved hybrids releasing.

The T 248, TB 367, A 665 inbred lines have been noticed only by the restoration abi-lity of *cms*-M and the maintainer *cms*-T, *cms*-C and *cms*-ES. Therefore, these lines could be utilized as indicators of *cms*-M type.

The observations performed on the restoration reaction of *cms*-C and *cms*-ES demonstrate the close relationship between the two representatives of C group.

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