Inheritance of Male Fertility Restoration for the Polima CMS System in *Brassica napus* L.

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INTRODUCTION

Several reports (Schuster and Michael 1976, Shiga 1976, Morice 1978, Buson 1980, Lefort-Buson 1982, Sernyk and Stefansson 1983 and Grant and Beversdorf 1985) have indicated that heterosis for seed yield in the 40% to 60% range occurs in spring and winter hybrids derived from crosses of rapeseed (*Brassica napus* L.) cultivars. These high levels of heterosis have stimulated interest in the development of hybrid rapeseed cultivars.

A pollen control system is mandatory to permit production of commercial quantities of hybrid seed in rapeseed (*Brassica napus* L.). These pollen control systems can be based on genic male sterility (Takagi 1970, Lee and Zhang 1983), recessive self-incompatibility (Thompson 1979) or on cytoplasmic male sterility (cms) systems (Thompson and Hughes 1986). Several male sterile cytoplasms have been discovered in recent years, including the *nap* (Thompson 1972), *ogu* (Bannerot *et al.* 1977) and *pol* (Fu 1981) cytoplasms.

Of these, the *pol* cytoplasm is considered by many Canadian rapeseed breeders to have the most immediate potential for use in hybrid rapeseed production. The development of a complete cms system using the *pol* cytoplasm requires the identification and production of maintainer and restorer lines in addition to the male sterile lines. Fan *et al.* (1986) reported that all *Brassica napus* L. cultivars tested in their research were partial or complete maintainers for the *pol* cytoplasm induced male sterility. Recently, a winter habit rapeseed (*Brassica napus* L.) strain called "Italy," known to be a restorer line for the *pol* cms system, was obtained from China. Additionally, a second winter habit rapeseed (*Brassica napus* L.) strain, "UM2353" (a University of Manitoba rapeseed accession) was discovered to be a restorer line for the *pol* cms system.

The objectives of this study were to characterize the male fertility restoration capabilities of Italy and UM2353 and to determine the inheritance of male fertility restoration from these two restorer sources for the *pol* cms system.

MATERIALS AND METHODS

Two BC8 (backcross eight) pol cms A lines derived from the cultivars Karat and Marnoo, (both spring habit cultivars), were crossed with Italy to generate F_1, BC_1, BC_2 and BC_3 generations and with UM2353 to generate F_1 and BC_1 generations. Appropriate generations were selfed to generate F_2, F_3, BC_1 selfed and BC_2 selfed generations for the Italy set of crosses and an F_2 generation for the UM2353 set of crosses.

All crosses were made in the greenhouse using bud pollination while selfed generations were obtained from bagged plants grown in the greenhouse. Italy, UM2353, F_2, BC and BC selfed generation seedlings were vernalized for four weeks at 4° C prior to transplanting to the greenhouse or field.

Observations on the degree of male fertility of all plants were recorded daily throughout the flowering period on newly opened flowers. All generations of materials were handled on a family basis. Chi-square tests for homogeneity of segregation ratios among families were calculated and found to be non-significant before the data for each generation was pooled. Chi square goodness of fit tests were calculated on the pooled data.

RESULTS AND DISCUSSION

The F_1 generations of the pol A Karat x Italy and pol A Marnoo x Italy crosses displayed only completely male fertile plants (Tables 1 and 2), indicating that Italy is pure breeding for one or more dominant pol cms system male fertility restorer gene(s). The virtually complete restoration of male fertility for the pol cms system effected by the Italy restorer gene(s) indicates that these restorer gene(s) have good potential for use in hybrid rapeseed seed production using the pol cms system.

The F_2 generations of the pol A Karat x Italy and pol A Marnoo x Italy crosses both segregated into a 3 male fertile:1 male sterile phenotypic ratio. F_3 families, derived from fertile F_2 plants, were of two types, (a) families which were pure breeding for male fertility and (b) families which segregated into a 3 male fertile:1 male sterile phenotypic ratio. These family types occurred in an approximately 1(a): 2(b) ratio for the pol A Karat x Italy cross, (Table 1), but in a 3(a): 1(b) ratio for the pol A Marnoo x Italy cross, (Table 2), due most probably to sampling error because of the small number of F_3 families studied.

The BC_1, BC_2 and BC_3 generations of both the pol A Karat x Italy and the pol A Marnoo x Italy crosses segregated into a 1 male fertile:1 male sterile phenotypic segregation ratio (Tables 1 and 2).

The BC_1 selfed and BC_2 selfed generations of both crosses segregated into a 3 male fertile:1 male sterile phenotypic segregation ratio (Tables 1 and 2).

These results clearly indicate that Italy contains a single dominant Mendelian gene for male fertility restoration in the pol cms system. This is the best possible situation for a potential restorer line in any cms system.

Turning to the second source of pol cms system male fertility restoration, namely UM2353, the F_1 generations of the pol A Karat x UM2353 and pol A Marnoo x UM2353 were all completely male fertile

(Tables 3 and 4), indicating that UM2353 is pure breeding for one or more dominant pol cms system male fertility restorer gene(s). The male fertility restoration was virtually complete, as seen in the Italy crosses.

The F_2 generations of the pol A Karat x UM2353 and pol A Marnoo x UM2353 crosses segregated into a 3 male fertile:1 male sterile phenotypic segregation ratio (Tables 3 and 4).

The BC_1 generations of these two crosses segregated into a 1 male fertile:1 male sterile phenotypic segregation ratio (Tables 3 and 4).

These results indicate that UM2353 contains a single dominant Mendelian gene for male fertility restoration in the pol cms system.

A series of crosses is presently being processed to determine if the restorer gene from Italy and the restorer gene from UM2353 are allelic.

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Table 1. Male fertility/sterility segregation ratios and Chi-square tests for selected generations of the pol A Karat x Italy cross.

Generation	Total No. of Plants Obs.	No. of Families	Observed Segregation Fertile	Observed Segregation Sterile	Expected Ratio	Chi-square	P
P_1 (pol A Karat)	100		0	100	-	-	-
P_2 (Italy)	90		90	0	-	-	-
F_1	54		54	0	-	-	-
F_2	4,034		3,012	1,022	3:1	0.24	0.50-0.75
F_3 (Families)	429	7	429	0	-	-	-
	466	13	348	118	3:1	0.03	0.75-0.90
BC_1	98		46	52	1:1	0.37	0.50-0.75
BC_2	495		241	254	1:1	0.29	0.50-0.75
BC_3	771		390	381	1:1	0.11	0.75-0.90
BC_1 selfed	1,538		1,146	392	3:1	0.20	0.50-0.75
BC_2 selfed	1,325		1,013	312	3:1	1.49	0.20-0.30

Table 2. Male fertility/sterility segregation ratios and Chi-square tests for selected generations of the pol A Marnoo x Italy cross.

Generation	Total No. of Plants Obs.	No. of Families	Observed Segregation Fertile	Observed Segregation Sterile	Expected Ratio	Chi-square	P
P_1 (pol A Marnoo)	85		0	85	-	-	-
P_2 (Italy)	77		77	0	-	-	-
F_1	64		64	0	-	-	-
F_2	3,597		2,648	949	3:1	3.67	0.05-0.10
F_3 (Families)	697	9	697	0	-	-	-
	288	3	220	68	3:1	0.30	0.50-0.75
BC_1	100		53	47	1:1	0.36	0.50-0.75
BC_2	2,186		1,100	1,086	1:1	0.09	0.75-0.90
BC_3	2,400		1,202	1,198	1:1	0.01	0.90-0.95
BC_1 selfed	3,645		2,711	934	3:1	0.76	0.30-0.50
BC_2 selfed	819		606	213	3:1	0.44	0.50-0.75

Table 3. Male fertility/sterility segregation ratios and Chi-square tests for selected generations of the pol A Karat x UM2353 cross.

Generation	Total No. of Plants Obs.	Observed Segregation Fertile	Observed Segregation Sterile	Expected Ratio	Chi-square	P
P_1 (pol A Karat)	40	0	40	-	-	-
P_2 (UM2353)	45	45	0	-	-	-
F_1	18	18	0	-	-	-
F_2	95	64	31	3:1	2.95	0.05-0.10
BC_1	102	49	53	1:1	0.16	0.50-0.75

Table 4. Male fertility/sterility segregation ratios and Chi-square tests for selected generations of the pol A Marnoo x UM2353 cross.

Generation	Total No. of Plants Obs.	Observed Segregation Fertile	Observed Segregation Sterile	Expected Ratio	Chi-square	P
P_1 (pol A Marnoo)	38	0	38	-	-	-
P_2 (UM2353)	47	0	47	-	-	-
F_1	20	20	0	-	-	-
F_2	98	68	30	3:1	1.65	0.10-0.20
BC_1	98	46	52	1:1	0.37	0.50-0.75