THE HETEROSIS, CYTOPLASMIC AND NUCLEAR EFFECT AND RECURRENT SELECTION OF CYTOPLASMIC MALE STERILE RESTORERS IN BRASSICA NAPUS L.

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INTRODUCTION

We began to study and breed self-incompatible (SI) lines, cytoplasmic male sterile (CMS) lines and their hybrids in 1971. Polima cytoplasmic male sterility (pol CMS) of *Brassica napus* was discovered in 1972 (Fu 1981). The SI lines 211, 271 of *B. napus* were bred in 1975 (Fu 1975; 1981). Prior to 1980 our work concentrated on the development of SI lines and their hybrids. Since 1980, the emphasis has been placed on the development of *B. napus* CMS lines and their hybrids. Several SI and CMS hybrids have now been developed and released. To determine the relative merits of the two pollen control systems, the yield of SI and CMS hybrids were compared.

Both nuclear and cytoplasmic genomes may have an effect on the heterosis of an F1 hybrid. It has been suggested that the nap CMS has negative effects on the heterosis of F1 hybrids and that the pol CMS does not (Guan 1986; Chen and Fu 1989). But the effect on the F1 of the restorer and maintainer nuclear genomes has not been previously considered. This paper gives additional information of effects on F1 hybrids of CMS cytoplasms and their restorers and maintainers.

The restoring genes (Rf) for pol CMS have been found in European varieties of *B. napus* (Chui *et al*. 1979; Liu and Fu 1987) and Chinese varieties of *B. juncea* and *B. campestris* (Fu and Yang 1987; 1990). We hope to group these Rf's into one random mating population (RMP) using the dominant genic male sterile (GMS) line Yi-3. Recurrent selection techniques will be used to enhance the fertility restoration of restorers and to improve their quality and agronomic characteristics.

MATERIALS AND METHODS

The yield of five pol CMS hybrids and two SI hybrids (Table 1), and the double high check cultivar, Ganyiu No. 5, were compared in a randomized complete block design with three replications, using plots 13m^2.

Two pol CMS lines (pol-003A and pol-864A) and two 75-3 CMS lines (75-3-003A and 75-3-864A) were obtained by using maintainers 003B and 864B as the recurrent male parents in six backcrosses to females with Polima A and 75-3A cytoplasm. Three restorers, RC88-6847, RC10 and RC96 developed at Huazhong Agricultural University, were used as pollinators to mate with the four CMS lines and their two common maintainers. These crosses produced 24 F1 hybrid strains (Table 2). These 24 hybrids were evaluated in 1990 in three row, 3m^2 plots arranged in a randomized complete block design with three replications. The growth period and disease resistance of the hybrids were investigated in the field. At maturity 10 plants were drawn at random from each plot, and the agronomic and economic characters of each plant evaluated. The mean of each character for each plot was used in a variant analysis.

The Random Mating Population (RMP) of pol CMS restorers was established by first using more that 30 double low restorers to pollinate the male sterile plants of the dominant GMS line Yi-3 A. Then these restorers were used in backcrosses with male sterile plants in F1. Finally the fertile plants were allowed to randomly pollinate the male sterile plants in the BC1 population. The Mo seeds were then harvested from the male sterile plants in the BC1. In the spring of 1990, 87 plants were randomly sampled to cross with pol CMS line 123A, and the fertility of each F1 was observed in the summer nursery in Kunming. The glucosinolate content of 94 plants from the Mo population was analyzed using the PdCL2 method; the erucic acid content was analyzed by the single-seed method.

RESULTS

The comparison of the yield of CMS hybrids and SI hybrids

The yield of five pol CMS hybrids and two SI hybrids was compared (Table 3). The yield of the double high CMS hybrid No. 1 was slightly lower (2.4%) than that of the check cultivar, Ganyiu No. 5, but the yield of the four single low CMS hybrids Nos. 2, 3, 4 and 5 were very significantly higher (20.72%, 14.77%, 18.56% and 12.43%; respectively) than that of the check. The yield of SI hybrid No. 6 is significantly higher (11.08%) than the check while the yield SI hybrid No. 7 was not significantly greater than the check. These comparisons suggested that these single low pol CMS hybrids and SI hybrids, bred in 1986, had higher seed yields than the CMS hybrid, bred ten years previously. Obviously, it is possible to find both CMS and SI hybrids that have strong heterosis and improved seed quality.

The effects of CMS cytoplasms, maintainer and restorer nuclei on the characteristics of F1 hybrids

The differences between aF1's(male sterile line x restorer) and bF1's (maintainer x restorer), which have the same nuclei and different cytoplasms, were compared as were differences between F1's produced with the same cytoplasm and maintainer but with different restorers, and F1's with the same cytoplasm and same restorers but different maintainers. All hybrids were evaluated using 12 characteristics. The results were as follows:

1. Effect of male sterile cytoplasms.

There were no significant differences between aF1's and bF1's for the 12 characteristics studied (Table 4). It is suggested that the two cytoplasms, pol, and 75-3 cytoplasm, do not negatively effect the F1 hybrids as compared with the normal cytoplasm.

2. Effect of two male sterile cytoplasms.

No significant differences were found between hybrids using pol CMS and 75-3 CMS cytoplasms for most of the characteristics examined (Table 5). These results suggested that pol CMS and 75-3 CMS effect F1's in the same way when their nuclear background is the same.

3. Nuclear effects of pol CMS and 75-3 CMS maintainers and restorers on plant-yield.

When the nuclear effects on F1 plant yield of two maintainers (003B and 864B) and three restorers (RC10, RC96 and RC6847) were analyzed, it was found that there were no significant differences among the three restorers. However, the two maintainers had significantly different effects on the yield of the F1's (Table 6). Maintainer 864B(A) produced F1's that yielded 33.67% more seed that those F1's maintained by 003B(A).

Investigations concerning the Random Mating Population of CMS restorers

Eighty-seven plants from the Mo generation of this RMP were chosen at random to pollinate the pol CMS line 123A in the spring of 1990. The fertility of all F1's was observed in the summer of the same year in Kunming. Of the 87 F1 progenies observed 24 had their male fertility completely restored, 22 were completely sterile and 41 were segregated fertile and sterile plants. These results suggest that the frequency of dominant Rf allels is about 0.5115 in the Mo population.

At maturity 94 plants of the Mo generation were harvested at random, and the glucosinolate content of their seed analyzed using the PdCL2 method. Eight plants with low glucosinolate (less than 35 µmol/g) were identified indicating that the frequence of the gene combination conditioning the low glucosinolate characteristic in this population was about 0.734.

The erucic acid content of 1140 seeds harvested randomly from male sterile plants in the Mo population were analyzed using the single-seed method. Four hundred and twenty-two low erucic acid seeds were found. Thus the frquency of the low erucic acid genes may be about 0.780.

DISCUSSION AND CONCLUSIONS

In the mid 1970s, we selected several SI hybrids which had a higher seed yield than the CMS hybrid that was developed about the same time by the Hunan Academy of Agricultural Science. By the 1980's, we had developed several CMS hybrids which had higher seed yields than the SI hybrids. Therefore, both the cytoplasmic male sterility and self-incompatibility could be equally important in the utilization heterosis in rapeseed. After a certain amount of research, hybrids with good qualities and greater heterosis in seed yield could be developed in both CMS and SI hybrids.

After combining quality characteristics and heterosis in our breeding programs, the ideal restorers we expected have not been found. The reason may be that by concentrating selection on the quality aspects and the restoring ability of CMS restorers rather than on economic characteristics we may have sacrificed yield heterosis. Recurrent selection within a RMP of CMS restorers may be an efficient way to improve pol CMS restorers.

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Table 1. The serial numbers of the crosses between CMS lines or maintainers and restorers of pol CMS and 75-3 CMS

Sterile lines or maintainer	Restorers		
	RC6847	RC10	RC96
75-3-003A	1	2	3
75-3-003B	4	5	6
pol-003A	7	8	9
pol-003B	10	11	12
75-3-864A	13	14	15
75-3-864B	16	17	18
pol-864A	19	20	21
pol-864B	22	23	24

Table 2. Seed yield of five CMS hybrids and two SI hybrids

Hybrid Number	Yield (kg/ha)	% over CK	Restoring fertility probability	Date of End Flowering
pol-Xiangai A x Huaie	2032.5	-2.4	90.10	4/11
pol-aA x RC10	2512.5	20.72	98.52	4/9
pol-aA x RC96	2389.5	14.77	95.16	4/10
pol-123A x RC10	2467.5	18.56	87.12	4/11
pol-123A x RC86	2340.0	12.43	89.10	4/9
SI-184 x Marnoo	2313.0	11.08	-	4/16
SI-352 x 81006	2160.0	5.05	-	4/12
Ganyou No. 5	2082.0	-	-	4/13

Table 3. Means of seed yield in grams per plant of F1s of the maintainers and restorers

Maintainers	Restorers			Xrc
	RC10	RC96	RC6847	
003B	12.45(b)	12.00(b)	11.27(b)	11.91(B)
864B	15.84(a)	16.53(a)	15.39(a)	15.92(A)
Xmr	14.16(a)	14.27(a)	13.33(a)	

Xrc: Mean seed yield per plant of maintainer over all restorers

Xmr: Mean seed yield per plant of restorer overall maintainers

Table 4: Reciprocal difference between hybrids in male sterile (aF_1's) and male fertile (bF_1's) cytoplasms for 12 characteristics

Characteristic evaluated	Differences between F1's (aF1-bF1)†											
	1-4	2-5	3-6	7-10	8-11	9-12	13-16	14-17	15-18	19-22	20-23	21-24
Days to flower	2.67*	0.67	-0.67	0.33	-1.00	-0.33	-0.67	0.67	0.00	-0.33	-0.33	-0.67
Plant height (cm)	-2.17	-0.64	0.93	2.70	-2.06	-8.47	0.04	-8.77	-4.00	0.30	-14.49*	-2.70
No. sec. branches	-0.74	-0.30	0.26	0.66	0.08	-0.14	-0.13	-0.20	0.41	0.47	-0.05	1.86*
Main inflor. l(cm)	-0.43	-1.35	-0.40	6.77	-1.64	-2.67	-0.53	-3.1	-1.91	-1.06	-9.69*	1.95
No. pods	0.07	3.77	3.84	5.23	-2.92	-6.72	4.17	-6.20	-4.53	0.73	17.89*	2.30
Pod density	0.01	0.07	0.06	0.01	-0.01	-0.06	-0.07	0.00	0.02	0.02	-0.02	0.01
Pod length (cm)	0.08	0.01	-0.07	-0.13	0.44**	-0.10	-0.04	0.14	-0.02	-0.20	-0.07	0.01
Seed/pod	1.01	1.13	-2.35	-1.80	2.42	-0.13	0.74	0.87	0.61	-1.92	0.90	1.30
Pod/plant	-28.85	-84.01	56.67	42.43	38.14	60.72	12.99	-30.40	-10.33	-59.29	-1.55	-42.05
Seed wt./1000 seed	-0.14	0.30	0.08	0.14	0.07	0.08	-0.02	0.08	-0.02	0.26	-0.12	0.04
Yield/plant g	-3.04	2.24	1.96	1.94	2.15	-2.90	1.30	-2.43	1.06	-1.35	-3.55	5.55
Sclerotinia index	6.69	7.99	14.92**	0.59	15.47**	13.37	10.82	6.82	-1.86	-0.81	-3.06	10.84

† Code numbers of genotypes identified in Table 1.

*, ** Significantly different at the .05 and .01 level, respectively.

Table 5: Difference between aF_2 hybrids produced with two different CMS cytoplasms, pol CMS and 75-3 CMS for 12 characteristics

Characteristic evaluated	Differences between F1's ($aF_1pol-aF_1$ 75-3)†						
	1-7	2-8	3-9	13-19	14-20	15-21	X
Days to flower	1.67	-0.66	-2.34*	-0.34	0.00	-0.33	0.33
Plant height (cm)	3.80	3.77	8.50	0.94	-1.20	-4.90	1.82
No. sec. branches	-0.04	-0.29	0.43	-0.67	-0.57	-0.95	-0.35
Main inflor. l(cm)	0.03	0.10	10.57**	-4.34	-2.60	-1.31	0.41
No. pods	-0.63	8.88	13.29	-4.56	1.36	0.91	3.21
Pod density	-0.01	0.11	0.03	-0.03	0.06	0.10	0.04
Pod length (cm)	0.11	0.25	0.24	0.07	0.25	0.10	0.09
Seed/pod	2.69*	-0.51	0.14	2.58	3.69*	0.38	1.50
Pod/plant	32.45	-25.37	25.96	-9.44	-2.58	-17.51	16.42
Seed wt./1000 seeds	0.04	-0.25	-0.13	-0.01	0.15	-0.13	-0.06
Yield/plant g	-1.43	-0.12	4.10	0.23	1.00	-1.74	0.34
Sclerotinia index	-0.77	-6.99	-2.03	10.57	10.46	-4.75	1.08

† Code numbers of genotypes identified in Table 1.

*, ** Significantly different at the .05 and .01 level, respectively.