RADISH CYTOPLASMIC MALE STERILITY IN RAPESEED: BREEDING RESTORER LINES WITH A GOOD FEMALE FERTILITY

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INTRODUCTION

Heterosis for seed yield in F1 hybrids of rapeseed has been reported by various authors (Grant and Beversdorf,1985; Lefort-Buson,1986; Brandle and McVetty,1989) and different systems of controlled pollination for hybrid seed production have been proposed.
Male sterile cybrids have been obtained through protoplast fusion between a rapeseed variety and a cytoplasmic male sterile line of rapeseed (with "Ogura" radish male sterility inducing cytoplasm) (Pelletier et al,1983).
No restorer was found in Brassica napus. The introduction of restorer genes from radish into rapeseed provided plants fully restored with one dominant restorer gene for the most interesting cybrids (Pellan-Delourme et al,1987; Pelletier et al,1987). However, the male fertility restoration was closely related with a decrease in seed set (Pellan-Delourme and Renard,1988). Observation of embryo sacs inside the ovules and correlation between number of seeds per pod and percentage of octonucleate embryo sacs indicated that low seed set could be attributed to a high rate of embryo sacs abortion.
During the last years, breeding was undertaken to try to eliminate the unfavourable radish information and to recover a better female fertility. At the same time, an isozyme marker was searched to make the selection easier.

MATERIAL AND METHODS

The origin of the restorer material has already been described in Pellan-Delourme and Renard (1988). Breeding of this material was continued through self pollinations, backcrosses with double low rapeseed line and testcrosses on cybrid male sterile lines. The restored plants were selected mainly on cybrid 27 and 58 cytoplasms.
In the fields, plants were choosen according to their pod development. Their female fertility was assessed by counting the number of seeds per pod. One sample of 50 pods was taken on each plant (25 pods on the primary branch and 25 pods on the secundary branches). In the greenhouse, the female fertility was estimated by the percentage of octonucleate embryo sacs after observation of cleared ovules with the technique described in Pellan-Delourme and Renard (1988). Five pistils were taken on each plant.
Isozyme studies were performed on segregating families using the technique described by Delourme and Foisset (1991).

RESULTS AND DISCUSSION

Figure 1 shows the evolution of the female fertility of the winter restored material during the last four years. The average number of seeds per pod was increased and some plants equalled the standards. In 1989, an improved family (R20) was selected with 19.6± 3.3 seeds per pod on average (Standards: X = 19.8 ± 3.2). The progeny of this family was studied in 1990. The average number of seeds per pod for the whole progeny was 15.1± 3.6 compare with 19.1 to 20.3 for the standards (Table 1). Four homozygous restored lines (R38,R39,R49,R52) were observed. The female fertility of these lines, of their backcrosses and testcrosses is shown in Table 1. The backcross of one of these lines (R39) and its testcross on a male sterile cybrid were not significantly different from the male fertile standards according to seed set.

Among the different isozyme systems studied, only PGI (Phosphoglucoisomerase) showed different patterns for restored and male sterile plants. The restored plants had two more bands compare to the sterile ones. This was explained by the presence in the restored plants of an allele of radish interacting with the alleles of rapeseed for Pgi-2 gene. Actually, this allele was found in some radish varieties.
To assess the tightness of the linkage between the restorer gene and radish Pgi-2 gene, crosses between heterozygous plants and maintainer or male sterile lines were studied. The presence of the restorer gene was correlated with the one of radish isozyme allele except for very few plants which were restored but did not possess the marker. The rate of recombination was lower than 1%.

The female fertility of the progeny of the plants which had lost the radish isozyme marker (R1-5-38,R52-14,R109) was observed in the greenhouse and was compared with the one of the best family previously described (R39) and the one of standard Samouraï (Table 2). The female fertility was assessed by the percentage of octonucleate embryo sacs which was well correlated with the number of seeds per pod (Pellan-Delourme and Renard,1988). Table 2 shows that the plants which have lost the radish isozyme marker had a higher female fertility than those which haven't lost it and even than those of R39 line. Their female fertility was equal to that of Samouraï. It has now to be checked in the fields but it could be explained by the fact that unfavourable radish genetic information has been eliminated.

Further work is needed to study the genetic structure of these plants and the inheritance of the restorer gene: in the progeny of heterozygous plants,segregation was not always 1:1. Anyway, in F1 hybrid seed production, restorer lines will be homozygous, transmission of the restorer gene will not thus be a problem.
These results have to be confirmed by experimenting the material in trials and by producing restored F1 hybrids
Now, the improvement of female fertility of the restorer material is very promising. It leads to a workable cytoplasmic male sterility system (Table 3).

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Figure 1: Evolution of female fertility of winter restorer material over four years.

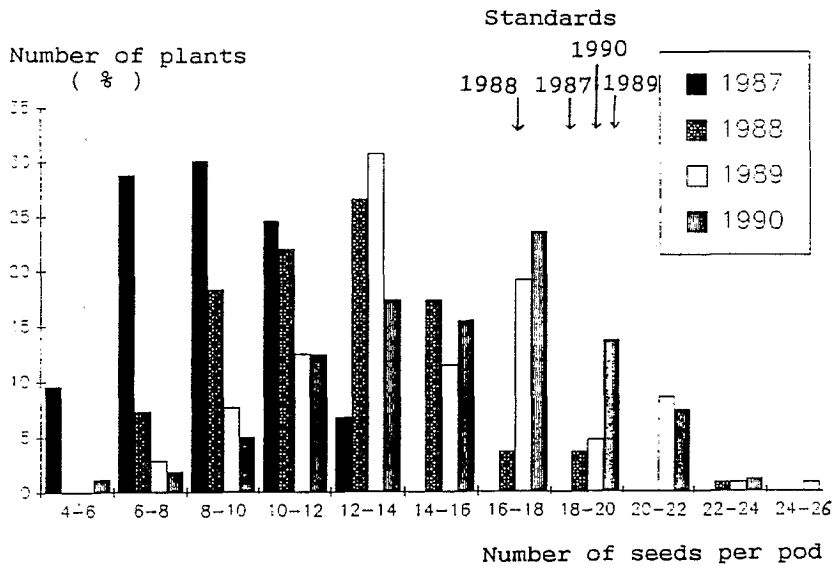

Table 1: Female fertility of the four homozygous line progeny observed in 1990.

Genotypes	Number of plants	Number of seeds per pod ($\bar{X}$ ± SE)
Darmor	6	20.3± 3.8
Bienvenu	6	19.8± 2.9
Samouraï	6	19.1± 3.6
R20 progeny	160	15.1± 3.6
Other progeny	64	10.2± 2.8
R38	10	14.3± 3.5
R38 BC(1)	10	16.6± 2.8
R38 TC(1)	5	16.5 ± 2.1
R39	20	16.6± 3.2
R39 BC	10	18.2± 1.1
R39 TC	5	20.1± 2.5
R49	20	11.6± 3.5
R49 BC	10	15.2± 3.0
R52	20	13.1± 2.5
R52 BC	10	14.5± 2.8
R52 TC	5	17.0± 0.8

(1): BC: Backcross with a maintainer line
TC: Testcross on a male sterile line

Table 2: Comparison of the female fertility of restored plants with or without the radish isozyme marker.

Genotypes	Number of plants	Percent of octonucleate embryo sacs ($\bar{X}$ ± SE)
Samouraï	2	92.5
R1-5-38 BC(1)	10	91.5 ± 3.8
R1-5-38 F2(1)	5	92.0± 2.5
R52-14 BC	9	89.9± 3.0
R52-14 F2	7	94.3± 4.8
R109 BC	6	92.8± 2.8
R109 F2	6	93.7± 2.1
R39 BC	13	79.9 ± 10.1

(1): BC: Backcross with a maintainer line
F2: Self pollination

Table 3: Characteristics of the improved cytoplasmic male sterility system.

	Original Ogura radish system	Improved radish system
Male sterile lines		
CMS stability	++	++
Photosynthesis	+/-	++
Nectaries	-	++
Female fertility	+	++
Maintainers	++	++
Restorers		
Genetics	many genes	one dominant gene
Male fertility	+/-	++
Female fertility	-	++