BREEDING DOUBLE LOW RESTORER LINES IN RADISH CYTOPLASMIC MALE STERILITY OF RAPESEED (*BRASSICA NAPUS* L.)

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ABSTRACT

Improvement of female fertility of restorer lines for the Ogu-INRA cytoplasmic male sterility has led to the obtention of R lines with a good productivity. This was accompanied by a better transmission of the restorer allele and a more regular meiotic behaviour. Linkage between male fertility restoration and glucosinolate content make the breeding of double low R lines more difficult.

INTRODUCTION

The cytoplasmic male sterility (CMS) originally found in by Ogura (1968) was transferred to *B.oleracea* and then to *B.napus*. Male sterile *B.napus* cybrids were then produced through protoplast fusion (Pelletier *et al*, 1983) to generate male sterile lines with a highly stable male sterility, improved nectar secretion and a high productivity (Pelletier *et al*, 1987). Fully restored plants with one dominant restorer allele introgressed from radish were selected on the best cybrid cytoplasms (Pelletier *et al*, 1987). However, the introduction of the restorer allele was accompanied by a large decrease in seed set (Pellan-Delourme and Renard, 1988). During the last years, improvement of the restorer material for the female fertility has been achieved. This success was possibly due to the elimination of radish genetic information. Isozyme studies performed on the improved restorer lines revealed the presence of a radish *Pgi-2* allele tightly linked to the radish restorer allele (Delourme and Eber, 1992). Specific molecular markers of radish have also been found around the restorer allele (Delourme *et al*, 1994). Therefore, some radish DNA still remains around the restorer gene. However, a few restored plants were found to have lost the radish *Pgi-2* allele.

This paper reports on the improvement of the female fertility of the restorer lines (R lines) and the consequences on the transmission of the restorer allele and on the meiotic behaviour of the restored plants. Progeny with or without radish *Pgi-2* allele are compared. Results of breeding for double low restorer lines are also discussed.

RESULTS

Improvement of the female fertility

The origin of the restored *B.napus* lines carrying the radish restorer allele has been described previously (Pellan-Delourme and Renard, 1988). Breeding of this marerial was continued through self pollinations, backcrosses with double low rapeseed lines and testcrosses on cybrid 58 male sterile lines. In 1989, an improved family was selected, giving rise to a progeny with a good female fertility (Delourme *et al*., 1991).

The female fertility of the winter restorer material has then been studied for several years and the number of seeds per pod of many homozygous restorer lines was shown to be significantly higher than the one of non improved controls and not significantly different from standard varieties (the number of seeds per pod of 60 % and 20 % of the R lines was equal or higher than that of 'Samourai' and 'Darmor', respectively). The female fertility of heterozygous restored plants was equal to the one of the control 'Samourai' but was lower than that of male sterile plants of the same progenies (Table 1). This result is similar to what is observed when comparing male sterile alloplasmic lines (on cybrid cytoplasm) to their male fertile recurrent lines.

Table 1: Number of seeds per pod (NSP) of heterozygous restored and male sterile plants from segregating progenies with 'Samourai' as reference.

Year	Number of R lines	NSP Samourai	NSP restored plants (% of Samourai)	NSP male sterile plants (% of Samourai)
1992	37	20.4	99.2	115.1
1993	56	18.2	99.2	110.9

The female fertility of restored progenies with or without *Pgi-2* allele of radish was then compared. No difference was observed when the restored plants were at the heterozygous stage. But the homozygous restored plants which have lost *Pgi-2* allele of radish showed a very poor seed set. This could be explained by the fact that the plants which have lost radish *Pgi-2* allele (PGI -) lack a rapeseed chromosomal segment. Studies are in progress to see if such PGI - material can nevertheless be used in F1 hybrid seed production.

Transmission of the restorer gene

Table 2 summarizes the transmission of the restorer allele from heterozygous plants through male (testcrosses) and female (backcrosses) gametes and in self pollinations in spring and winter restorer material over many years. The improvement of the female fertility was accompanied by a much more regular transmission of the restorer allele, especially on the female side. The transmission of the restorer allele through the female gametes remained higher than through the male ones but was greatly regularized. Furthermore, the transmission of the restorer allele was better in the material with *Pgi-2* allele of radish than in the material which has lost *Pgi-2* allele of radish.

Table 2: Transmission of the male fertility restorer allele in progenies with improved or non improved female fertility and with (PGI +) or without (PGI -) Pgi-2 radish allele..

Female fertility	Year	PGI	Backcrosses	Testcrosses	Self pollinations
non improved	1988 - 90	+	92.5[a] (50)[b]	48 (50)	93 (75)
improved	1991 - 94	+	62 (50)	47 (50)	76 (75)
		-	45 (50)	38 (50)	61 (75)

a: Mean percentage of restored plants b: Expected percentage of restored plants

Thus, in the material with a good female fertility and with *Pgi-2* allele of radish most of the progenies have segregation ratios not significantly different from expected mendelian ratios with one dominant restorer gene.

These results could be related to the meiotic behaviour observed in the restored plants. When the female fertility was very poor, the meiotic behaviour of the plants was disturbed with a high amount of univalents and multivalents (Pellan-Delourme and Renard, 1988). In the improved material, the meiotic behaviour has been greatly regularized but from time to time, abnormal meiotic behaviour can be observed, which leads to abnormal segregation and sometimes to aneuploid plants. A selection must then be made during R lines breeding to eliminate badly segregating progenies.

Breeding double low R lines

Backcross schemes have been initiated to breed double low R lines. Preliminary results seemed to indicate that it was difficult to get R lines with a glucosinolate content lower than 25

µmoles. A linkage between male fertility restoration and glucosinolate content was observed both in PGI + and PGI - material (Fig.1).

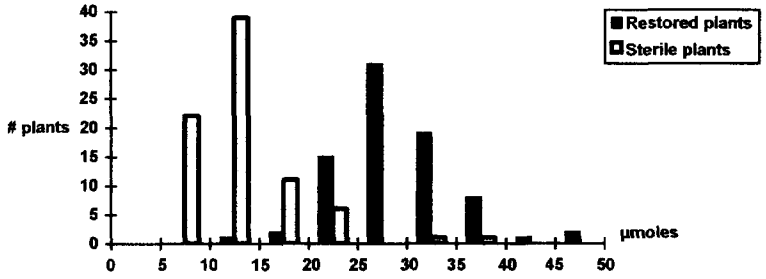

Figure 1: Glucosinolate content of heterozygous restored and male sterile plants from backcross progenies (B3F1 and B5F1).

Nevertheless, some double low restored plants were obtained in spring as well as in winter material, indicating that it is possible to break the linkage. However, the glucosinolate content and meiotic behaviour of the selected plants must be checked next year to be sure to produce stable double low R lines.

CONCLUSION

The selected double low R lines will be used to produce restored F1 hybrids or mixed F1 hybrids, depending on the glucosinolate content and relative yield of these two varietal types.

To make *Ogu*-INRA CMS system more easily workable in double low R lines breeding, more radish genetic information should be eliminated. Different strategies are under development to reach this target such as recurrent selection in double low restored populations to stimulate efficient recombination or gene tagging to try to isolate only the restorer allele.

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