# MALE FERTILITY RESTORATION IN BRASSICA NAPUS WITH RADISH CYTOPLASMIC MALE STERILITY

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Radish (Raphanus sativus) cytoplasmic male sterility (OGURA,1968) has been transferred to Brassica oleracea and Brassica napus (BANNEROT et al,1974).Resultant male sterility is highly stable but it is accompanied by chlorophyllous deficiency,absence of nectar secretion ,and absence of restorer genes in rapeseed(ROUSSELLE,1982).The problem of chlorophyllous deficiency has been solved by protoplast fusion,which has also improved nectar secretion(PELLETIER et al,1983).Since restorer genes are present in radish,introduction to rapeseed was attempted using an intergeneric cross of a male-sterile rapeseed with 'Ogura' type cytoplasm,and a Raphanobrassica carrying restorer genes(HEYN,1976).The present study of male fertility restoration concerns the progeny of that cross.Twelve families (eleven winter type,one spring type) were selected by crossing with a male-sterile 'Ogura' type line,and self-pollination of restored plants (BANNEROT,BOULIDARD,pers. comm.).Progeny of the eleven winter type families were followed through self-pollination and crossing with three cultivars of winter rapeseed.After crossing with a spring rapeseed line and selection of restored plants ,the spring type family was used as pollinator to compare restoration on the original 'Ogura' type radish cytoplasm,and four cytoplasms obtained from protoplast fusion ( cybrids 27,58,77 and 118; PELLETIER et al,1983).

## A.QUALITY OF RESTORATION

Three plant types were distinguished from visual assessments of male fertility for the twelve families and their progeny:

- fertile plants,
- plants of intermediate fertility,either with thin stamens and low pollen production,or with appreciable between flower variation in fertility,
- male-sterile plants with reduced or pistilloid stamens.

Two types of cytoplasm were distinguished in relation to male fertility:

- 'Ogura' cytoplasm and cytoplasms from cybrids 77 and 118,for which plant fertility could be divided into the three classes described above,
- cytoplasms from cybrids 27 and 58 for which plant fertility was either complete or nul,restored plants being completely fertile,none being intermediate.

## B.DETERMINISM OF RESTORATION

Two classes were distinguished:

- male-sterile plants without restorer allele,
- male-fertile or partly male-fertile plants with one or several restorer alleles.

Study of winter and spring type progeny showed:

1) An environmental effect on the expression of male fertility restoration,or on differences in seed germination,depending on presence or absence of restorer genes: For a same F1 cross,proportions of male-sterile plants was higher in the field than in the greenhouse,segregations in the latter corresponding with normal ones.

2) Differential transmission of restorer genes by male and female gametes: Comparisons of progeny of the same plants,obtained by self-pollination and crossing with rapeseed cultivars under identical conditions showed some contradictory features.These were confirmed from study of a monogenic character also derived from radish,white flower colour,and differentially transmitted by male and female gametes.Plants studied also showed low female fertility,which might account for irregularities in character transmission on the female side.

3) A difference between cytoplasms: In the greenhouse,the proportion of male-sterile plants in the progeny with cybrids 27 and 58 cytoplasms supports the hypothesis of one restorer gene ,whereas the proportion in plants with cybrids 77 and 118 cytoplasms is in favour of two.Segregation in field plants does not suggest any specific hypothesis (except for cybrid 118) but the proportion of male-steriles in plants with cybrids 27 and 58 cytoplasms is lower than for those of plants with cybrids 77 and 118 cytoplasms (Table 1).This is in agreement with absence of plants of partial male fertility for cybrids 27 and 58 cytoplasms and suggests that

restoration may be simpler for the latter than for those of cybrids 77 and 118.These differences correspond to those found at the level of mitochondrial genome (PELLETIER et al,these proceedings) and support the hypothesis that the molecular basis of cytoplasmic male sterility in rapeseed is to be found at that level.

## C.STUDY OF FEMALE FERTILITY

Female fertility was measured as:
- percentage of ovules with octonucleate embryo-sac,
- number of seeds per pod.

Plants with low female fertility had a certain percentage of embryo-sacs stopped at uninucleate,or,less often,at bi- or tetranucleate stages.Table 2 shows that:

1) female fertility is always lower for plants derived from restorer material than for standards;
2) low female fertility is mainly due to high levels of ovule abortion (high correlation between number of seeds per pod and percent of octonucleate ovules);
3) low female fertility and male fertility restoration are linked.Male-sterile plants in the F1 (from crosses between male-steriles and restored plants) that did not carry restorer genes had high female fertility.Amongst plants obtained from self-pollination or backcrossing with rapeseed,only plants which eliminated restorer genes had high female fertility.In the progeny from winter 1984-85 (obtained from F1 plants after selfing and crossing with rapeseed) variation in female fertility was considerable,notably for male-steriles.Some plants had very low female fertility while for others this was similar to standards,the latter accounting for differences between male-sterile plants on the one hand and male-fertile and partly fertile plants on the other.

Study of meiotic behaviour in 68 plants from the twelve families showed that all possessed 2n=38 chromosomes,as for rapeseed.However,none had regular meiotic behaviour with 19 bivalents (PELLAN-DELOURME,1986).

Chromosome number was reduced to that of rapeseed during the different backcrosses by progressive elimination of radish chromosomes.Presence of multivalents implies that part of radish genome may still have been conserved as fragments of translocated chromosomes,although plants had only 38 in all.The amount

of genetic material from radish so introduced may be too large and be responsible for reduced female fertility.

## CONCLUSION

This study shows that radish restorer genes can ensure restoration in rapeseed.Genetic determinism of restoration in some cybrids may be simpler than for 'Ogura' type radish cytoplasm.However,introduction of restorer genes to rapeseed was accompanied by lowered female fertility.The restorer material,in its current genetic state,cannot be used to establish F1 hybrids.The reduction in such hybrids largely exceeds any improvement from heterosis.The aim,must now be to provoke recombination between restorer genes and radish genetic information responsible for lowered female fertility.

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Table 1: Proportion of male-sterile plants in the progeny of crosses between restored plants and male-sterile plants with 4 different cytoplasms.

| Cytoplasm | Greenhouse | | Field | |
|---|---|---|---|---|
| | Number of plants | Percent of steriles | Number of plants | Percent of steriles |
| Cybrid 27 | 146 | 48,6 | 243 | 65,0 |
| Cybrid 58 | 158 | 51,9 | 202 | 69,8 |
| Cybrid 77 | 114 | 76,3 | 300 | 85,3 |
| Cybrid 118 | 102 | 78,4 | 323 | 77,7 |

Table 2: Female fertility in the progeny of restored plants

| Progeny | Percent of octonu-cleate ovules | | Number of seeds per pod | |
|---|---|---|---|---|
| | Average | Relative to the standards | Average | Relative to the standards |
| WINTER 1984-85 | | | | |
| Standards | 96,8 | 100 | 21,7 | 100 |
| Partly and fully restored plants | 26,6 | 27,5 | 5,8 | 26,9 |
| Male-sterile plants | 46,7 | 48,2 | 9,0 | 41,5 |
| SPRING 1985 | | | | |
| Standards | 95,1 | 100 | 21,0 | 100 |
| Male-fertile F1 plants | 29,8 | 31,3 | 8,5 | 40,4 |
| Male-sterile F1 plants | 71,2 | 74,9 | 19,3 | 91,8 |