TRANSFER OF RADISH CMS-RESTORER GENE INTO *BRASSICA NAPUS* BY INTERGENERIC PROTOPLAST FUSION

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Abstract

To develop a pollination system for hybrid seed production in oilseed rape, we introduced a restorer gene from Kosena radish into cms cybrid of *B. napus* cv. Westar by asymmetric protoplast fusion. Three hundreds of fusion products could be regenerated and six of them restored male-fertility. All the male fertile and male sterile plants except one fertile plant had the cms related gene (orf125) surveyed by PCR. A fertile restored plant had 2n=47 chromosomes. By two backcrosses to the *B. napus* cms cybrid, BC2 plants showed *B. napus* chromosome number (2n=38). In the BC3 generation, no female sterility was observed and the introduced restorer gene showed single dominant inheritance in *B. napus*.

Introduction

Programs for commercial production of hybrid seeds (F1) in oilseed rape have been pursued over the past two decades. Several types of cms-restorer genes have been introduced in *B. napus*.

Recent progress in somatic hybridization had allowed the introduction of not only cytoplasmic traits such as cms cytoplasm but also of a limited amount of nuclear genes into recipient cells. We have successfully introduced Kosena cms derived from a cultivar of Japanese radish into *B. napus* by donor-recipient protoplast fusion (Sakai and Imamura 1992) and produced the cms lines. The lines showed a stable cms phenotype under various nuclear background in different temperature from 10℃ to 30℃.

In this report, we describe introduction a restorer gene (Rf) against Kosena cms by asymmetric protoplast fusions between the *B. napus* cms cybrid and the R. sativus restorer line. We also describe the agricultural characteristics of the restored plants and backcrossed progeny.

Results

Introduction of a restorer gene into *B. napus* by asymmetric cell fusion

The radish Rf line was isolated from natural population of Kosena radish (Sakai and Imamura 1992). The radish mesophyll protoplasts were x-irradiated at 60KR and fused with iodoacetamide (IOA) treated hypocotyl protoplasts of *B. napus* cms cybrids. The *B. napus* and radish protoplasts were mixed at a 2:1 ratio. Three hundred plants could be regenerated and six out of 300 plants restored male fertility. The fertile plants shed functional pollen grains and the percentages of morphologically normal pollen grains were ranged from 8% to 83%.

We surveyed a Kosena cms-related mitochondrial gene (orf125) by PCR. All the male-sterile and male-fertile plants analyzed had the cms related gene except one fertile plant, suggesting that the remaining five fertile plants have the Rf gene. The test cross of the fertile line to a *B. napus* cms cybrid confirmed that the observed restoration of male fertility was caused by introduced of the Rf gene in the cms cybrid.

Characterization of *B. napus* Rf line

A fertile R0 plant had 2n=47 chromosomes. After backcrosses to *B. napus* cms cybrid, *B. napus* chromosome number (2n=38) of the BC2 generation was observed (Fig.1). In BC3 generations, male-fertile and male sterile plants were segregated in a 1:1 ratio, indicating that single dominant restorer gene was introduced and integrated in the B. napus chromosome (Fig.1). Seed set of the Rf line was compared to that of *B. napus* variety (Table 1). No difference was observed in the number of seeds per pod between B. napus varieties and BC4 plants of Rf lines in a greenhouse conditions (Fig. 2). In summer field 1994, no yield penalty was observed in the Rf lines (Table 1). Taken together these results, yield penalty observed in the introgression of the Rf gene against Ogura cms cytoplasm (Pellen-Delourme and Renard 1988) was bypassed in the fusion derived Rf lines.

The elimination of the additional radish chromosomes was observed in BC2 plant (Fig. 1). In our experiments, complete loss of introgressed radish chromosomes in intergeneric hybridization was required at least five backcrosses to *B. napus* (data not shown), suggesting that x-irradiation to radish cells prior to the cell fusion resulted in the stimulation of the chromosome elimination from the fusion products.

Conclusion

We successfully introduced a Rf gene against Kosena cms by asymmetric protoplast

fusions. After the three times of crossings followed by the cell fusion, a Rf line without yield penalty could be established. The developed cms-Rf system should be available for the commercial production of F1 seeds in oilseed rape.

References

Sakai,Tand Imamura, J (1992) Alteration of mitochondrial genomes containing *atpA* genes in the sexual progeny of cybrids between *Raphanus sativus* cms line and *Brassica napus* cv. Westar. *Theoretical and Applied Genetics* **84**:923-929

Pellen-Delourme, R and Renard, M (1988) Cytoplasmic male sterility in rapeseed (Brassica napus L.): female fertility of restored rapeseed with Ogura and cybrids cytoplasms. *Genome* **30**:234-238

Fig. 1 Schematic representation of the transfer of Kosena Rf gene into B. napus by asymmetric fusion

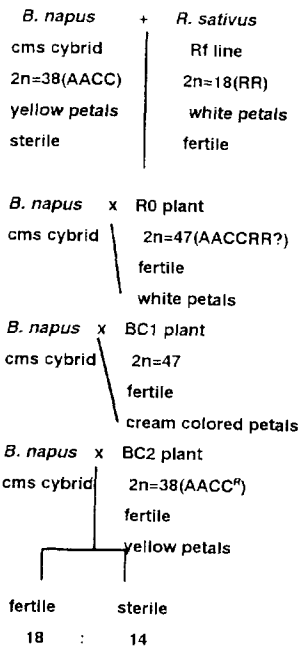

Table 1 Yield of the *B.napus* Rf lines on field (Hokkaido, Japan) 1994 summer

lines	Numbers of pods per plant	yields per plant(g)**
Rf lines		
71BC3-1	263(20)*	13.4
71BC3-2	309(12)	14.3
Variety		
Westar	205(5)	12.9
Celebra	283(5)	12.9
Vanguard	222(5)	11.5

* : () indicated the number of plants tested.
**:average yield per a plant of the tested plants were scored.

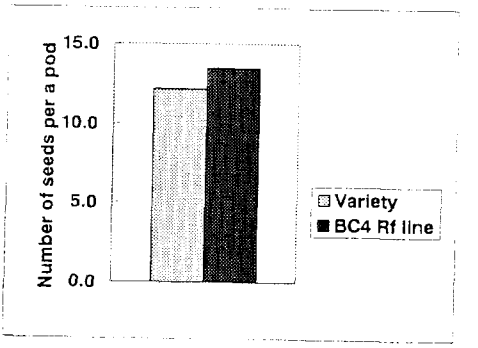

Fig.2 Average number of seeds per pod of Rf lines (BC4) and control varieties.
Two plants of BC4 Rf lines and seven plants of control varieties were grown in the growth chamber under 22C, 12h light conditions. Seeds in 20 to 70 pods per each samples were scored.