Mem. Fac. Agr. Kagoshima Univ., 22, 69–72, 1986

Transfer of Cytoplasmic Male Sterility from Tetraploid Radish, *Raphanus sativus* L., to *Raphanobrassica* by Means of Successive Backcrosses

Hiroshi OGURA, Takeomi ETOH and Tomio JOHJIMA
(*Laboratory of Vegetable Crops*)
Received for Publication September 9, 1985

Introduction

In the production of hybrid seeds and breeding, aiming the effective utilization of male sterility, recently, various studies have been carried out on the cytoplasmic or the cytoplasmic genetic male sterilities in several cultivating plants, including such autogamous plants as rice-plant and wheat as well as allogamous plants[5,12].

As a system of hybrid seed production in *Brassica* and *Raphanus*, male sterility is to be regarded as an economically valid alternative to self-incompatibility.

The male sterility which was concerned with a single recessive gene in radish was first reported by Tokumasu[13]. Then a different type of male sterile radish was found by Ogura, and its inheritance mode was shown to be concerned both with the cytoplasm and with the recessive gene[10]. This sterility was ascertained to have been manifested on the tetraploid forms brought forth by colchicine treatment from the diploid male sterile radishes[11].

In the process of introduction of this male sterility to European radish, *Raphanus sativus* L., it was found by Bonnet that this male sterility was concerned with the cytoplasm and at least with plural genes[3,4]. The transfer of this male sterility from *R. sativus* to *Brassica oleracea* was tried by Bannerot *et al.*, but it was resulted in failure because of the impropriety in the function of the nucleus of *B. oleracea* in the radish cytoplasm[1,2].

Materials and Methods

The parent plants used here included normal and male sterile tetraploid radishes ($2n=4x=36$), *R. sativus* L., and *Raphanobrassica* ($2n=36$). Tetraploid forms of radishes were brought forth by colchicine treatment from the normal and the male sterile diploid radishes, respectively. *Raphanobrassica* was a strain derived from offsprings of the cross, carried by Fukushima in 1935[6], between *R. sativus* L. cultivar Harumachi and *Brassica alboglabra* Bailey. Since then it has been maintained under the confirmation of chromosome number $2n=36$.

Before the performance of crosses, all the opened flowers and young flower-buds were removed from the selected inflorescences, and the large flower-buds in the stages from just before to a few days before openings were emasculated, and fresh pollen grains of the inflorescences which had been covered previously with paraffin paper bags were pollinated to stigmatic surface. The pollinated flower-buds were protected from the contamination with paraffin paper bags for at least one week.

It is well known that the cross between *Raphanus* and *Brassica* is successful only when the former species is adopted as the maternal parent, but it is otherwise in case of the reciprocal crossing[6–9]. In this study, *Raphanobrassica* was adopted as paternal parent and mated with normal and male sterile tetraploid radishes, and the successive backcrosses were carried out for more than eight years.

Results and Discussion

The viable pollen grains with the number less numerous than those in their parent were usually produced by the offsprings derived from the crosses between the normal tetraploid radish adopted as maternal parent and *Raphanobrassica* adopted as paternal parent (Fig. 6). Among the F_1 plants of the above mentioned cross, considerable differences were observed in the producibility of the viable pollen grains.

The viable pollen grains ranging from 20 to 25 percent were produced by the plants derived from the first backcross made to the F_1 hybrid with *Raphanobrassica*; n. BC_1 (Table 1). The gradual increasing of the viable pollen grains percentage was occasioned in accordance with the progression of the successive backcrosses, attaining to the percentage which was almost same as that of normal *Raphanobrassica* at n. BC_4 to n. BC_6.

Table 1. Percentages of pollen grains stained normally by acetocarmine

Strains and cross combinations	Percentage
Normal tetraploid radish.	74.1
Male sterile tetraploid radish.	0.0
Raphanobrassica.	91.6
F_1 hybrid of normal tetraploid radish × *Raphanobrassica*.	61.3
First backcross of the F_1 mentioned above with *Raphanobrassica*; n. BC_1.	24.8
Eighth backcross of the F_1 mentioned above with recurrent *Raphanobrassica*; n. BC_8.	87.9
F_1 hybrid of male sterile tetraplied radish × *Raphanobrassica*.	0.0
Eighth backcross of the F_1 mentioned just above with recurrent *Raphanobrassica*; s. BC_8.	0.0

On the other hand, in the occasion when the male sterile tetraploid radishes were adopted as maternal parent, all of the offsprings failed to produce viable pollen grains, showing male sterility. Manifestation of this sterility was noted in all the derived plants, from F_1 to the offsprings of the 8th backcross in this cross-combinations s. BC_8 (Fig. 5). On the other hand, the female function was kept always normal, and production of seeds was induced when they were pollinated with the pollen of *Raphanobrassica* (Fig. 2).

In the male sterile plants derived from the crosses mentioned above, degeneration of pollen began at the early developing stage of microspore. At the earlier stages before the formation of tetrad, no difference was observed between the normal and the male sterile plants. The pollen degeneration occurred suddenly and it might be anticipated from the abnormality of microspore, such as emptiness, shrinking or small size, as well as from the difficulty in being stained by acetocarmine.

By these results it was suggested the possibility that this male sterility might fundamentally be concerned with the cytoplasm of male sterile tetraploid radishes. Moreover it was suggested that for the gene concerned in the pollen-formation of *Raphanobrassica* it is not possible to operate its function effectively in the cytoplasm of the male sterile radishes.

Summary

Some crosses were carried out between the normal and the male sterile tetraploid Japanese radishes (2n=4x=36), *Raphanus sativus* L., and *Raphanobrassica* (2n=36). Tetraploid radishes were brought forth by means of colchicine treatment from the normal and the male sterile diploid radishes, respectively, and *Raphanobrassica* was synthesized from *R. sativus* L. and *Brassica alboglabra* Bailey.

Successive backcrosses of tetraploid radishes with *Raphanobrassica* have been carried out for more than eight generations.

In the occasion when the male sterile tetraploid radishes were adopted as maternal parent, male sterility was shown by all of the offsprings, while the female function of those was kept normal, producing seeds in the occasion when pollinated with pollen of *Raphanobrassica*. Obtaining of a male sterile line of *Raphanobrassica* was made possible by the performance of the successive backrosses mentioned above.

Acknowledgment

The authors wish to express their gratitude to Professor Ken-ichi Arisumi of Kagoshima University for suggestions made in the preparation of the manuscripts. They are also indebted to the members of Laboratory of Vegetable Crops of Kagoshima University for their much help in carrying out the experiments.

References

1) Bannerot, H., Boulidard, L., Cauderon, Mme Y. and Tempe, J.: Transfer of cytoplasmic male sterility from *Raphanus sativus* to *Brassica oleracea*. *Proc. Eucarpia Meeting-Cruciferae,* 52–54 (1974)
2) Bannerot, H., Boulidard, L. and Chupeau, Y.: Unexpected difficulties met with the radish cytoplasm in *Brassica oleracea*. *Eucarpia Cruciferae Newsletter,* 2, (1977)
3) Bonnet, A.: Introduction et utilisation dúne stérilité mâle cytoplasmique dans des variétés précoces européennes de radis, *Raphanus sativus* L.. *Ann. Amélior. Plantes,* **25**, 381–397 (1975)
4) Bonnet, A.: Breeding in France of a radish F_1 hybrid obtained by use of cytoplasmic male sterility. *Eucarpia Cruciferae Newsletter*, **2**, 5 (1977)
5) Edwardson, J. R.: Cytoplasmic male sterility. *Bot. Rev.*, **36**, 341–420 (1970)
6) Fukushima, E.: Cytogenetic studies on *Brassica* and *Raphanus*. 1. Studies on the intergeneric F_1 hybrids between *Brassica* and *Raphanus*. *J. Dept. Agr. Kyushu Impr. Univ.*, **7**, 281–400 (1945)
7) Karpechenko, G. D.: Hybrids of *Raphanus sativus* L. × *Brassica oleracea* L.. *J. Genet.*, **14**, 375–396 (1924)
8) McCollum, G. D.: Sterility in successive backcrosses of *Raphanobrassica* (2n=4x=36) with recurrent *Brassica oleracea* (2n=2x=18). *Can. J. Genet. Cytol.*, **21**, 479–485 (1979)
9) McNaughton, I. H.: Synthesis and sterility of *Raphanobrassica*. *Euphytica*, **22**, 70–88 (1973)
10) Ogura, H.: Studies on the new male-sterility in Japanese radish, with special reference to the utilization of this sterility towards the practical raising of hybrid seeds. *Mem. Fac. Agr. Kagoshima Univ.*, **6**, 39–78 (1968)
11) Ogura, H. and Etoh, T.: Manifestation of male sterility in autotetraploids derived from diploid male sterile radishes (*Raphanus sativus* L.). *Mem. Fac. Agr. Kagoshima Univ.*, **11**, 57–60 (1975)
12) Pearson, O. H.: Nature and mechanisms of cytoplasmic male sterility in plants: a review. *HortScience,*

16, 482–487 (1981)

13) Tokumasu, S.: Male-sterility in Japanese radish (*Raphanus sativus* L.). *Sci. Bull. Fac. Agr. Kyushu Univ.*, **13**, 83–89 (1951)

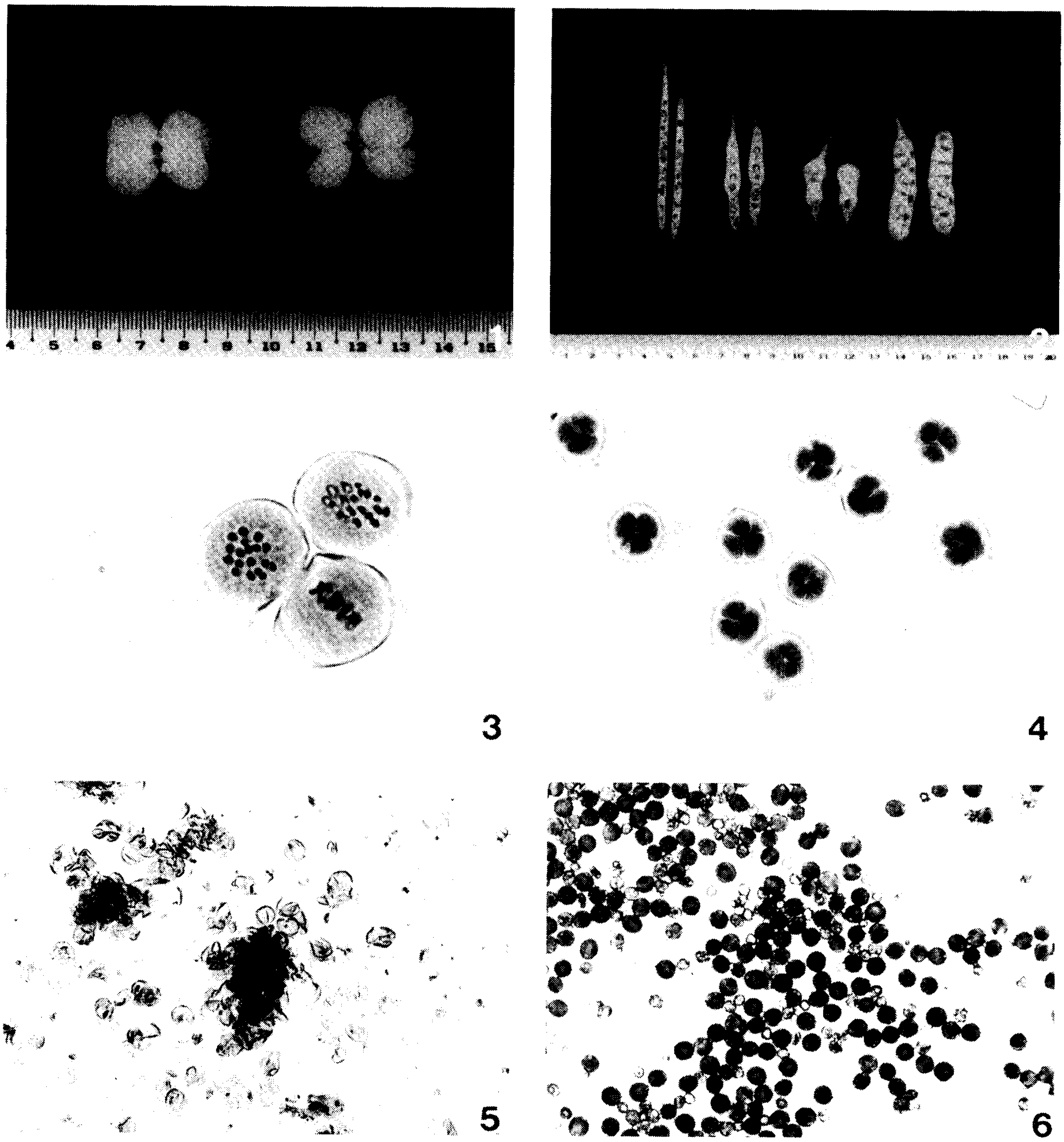

Fig. 1. Male sterile (left) and normal flower (right) of *Raphanobrassica*.

Fig. 2. From left to right longitudinal sections of pods of tetraploid *Brassica alboglabra*, *Raphanobrassica*, F_1 hybrid of tetraploid radish × *Raphanobrassica*, and tetraploid radish.

Fig. 3. Metaphase-I of meiosis of male sterile *Raphanobrassica*; s. BC_8.

Fig. 4. Tetrads of male sterile *Raphanobrassica*; s. BC_8.

Fig. 5. Degenerated microspores of male sterile *Raphanobrassica*; s. BC_8.

Fig. 6. Pollen graines of F_1 hybrid of normal tetraploid radish × *Raphanobrassica*.