Short Communication

Induction of Male Sterility in Hybrids between Japanese Wild and Cultivated Radishes (*Raphanus sativus* L.)

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To determine the origin of Ogura cytoplasmic male sterility in radish (*Raphanus sativus* L.), crossing experiments were conducted between Japanese wild and cultivated radishes. The mitochondrial (mt) DNA type and anther development of the F₁ plants were analyzed. In the crosses, wild radishes (*R. sativus*) collected from two locations in Japan (Kushikino and Tomioka) were used as female parents, and, four cultivars (Uchiki-Gensuke, Aokubi-Miyashige-Oonaga, Kangoshi-Tokinashi and Comet) as male parents. The mtDNA type of the hybrids was checked by the PCR-aided assay for the *atp6* region in which one of the rearranged sites between Ogura and normal mtDNA was located. Male fertility was evaluated by observing the anther development.

Ogura-type mtDNA was detected in all the hybrids between 'Kushikino' and cultivars, whereas both Ogura- and normal-type mtDNA were present in the hybrids between 'Tomioka' and cultivars. Half of the plants obtained in the cross between 'Kushikino' and each of the three cultivars (Uchiki-Gensuke, Aokubi-Miyashige-Oonaga and Kangoshi-Tokinashi), showed a male sterility, whereas all the hybrid plants using 'Comet' as a pollen parent showed a normal fertility. 'Comet' restored the fertility when it was crossed with 'MS-Gensuke' which had the Ogura male sterile cytoplasm. These findings suggest that the Ogura cytoplasm may have originated in the wild Japanese radish.

KEY WORDS: *Raphanus sativus* L., wild radish, Ogura cytoplasm, male sterility, mitochondrial DNA, PCR.

Introduction

The Ogura male sterile cytoplasm was detected in Japanese radish (*Raphanus sativus* L.; OGURA 1968), and has been extensively studied for practical use in radish as well as various *Brassica* crops (SHIGA 1980; KAO *et al.* 1991). However, the origin of this cytoplasm is poorly documented. MAKAROFF *et al.* (1988; 1989) first showed that rearrangements including each of *atpA*, *atp6* and *coxI* genes occurred between Ogura and normal mitochondrial (mt) DNAs. Their findings prompted us to study the origin of the Ogura cytoplasm by polymerase chain reaction (PCR) using the differences in mtDNA organization as molecular markers. By this approach, we recently observed that the Ogura-type mtDNA was widely distributed in Japanese wild radishes, whereas Japanese cultivars tested had the normal-type mtDNA (YAMAGISHI and TERACHI 1994).

To obtain further information on the origin of the Ogura cytoplasm, crossing experiments were conducted between the wild radishes and the cultivars, in an attempt to induce male sterility in the progenies of wild radish having the Ogura-type mtDNA. We report here the

Received July 30, 1993.

Accepted September 24, 1993.

appearance of male sterile plants among the F₁ hybrids between Japanese wild radishes and the cultivars.

Materials and Methods

Wild radish plants collected in 'Kushikino' (Kagoshima Prefecture) and 'Tomioka' (Kumamoto Prefecture) were crossed with the following four cultivars; 'Uchiki-Gensuke', 'Aokubi-Miyashige-Oonaga', 'Kangoshi-Tokinashi' and 'Comet'. 'MS-Gensuke', which has the Ogura cytoplasm and exhibits male sterility, was also crossed with the cultivars for comparison. Among the cultivars used as male parents, 'Uchiki-Gensuke' is a maintainer of the sterility.

Total DNA isolated from a young leaf of the hybrids was used as a template for PCRs in which the mitochondrial *atp6* region was amplified. The region was known to be rearranged in the Ogura mtDNA relative to normal mtDNA. By the PCRs, a 331 bp DNA fragment should be amplified in radishes with normal mtDNA, whereas a 546 bp DNA fragment should be yielded in the plant with the Ogura-type mtDNA. The details of the DNA isolation and the PCR procedures were described by YAMAGISHI and TERACHI (1994).

After a leaf was harvested for DNA isolation, the hybrid plants were exposed to a low temperature (5°C) for one month, and then returned to the greenhouse where the temperature was higher than 15°C. Anther development of the flowering hybrids was observed in February and March, 1993, and the hybrid plants were classified as either male sterile or male fertile.

Results and Discussion

All the F₁ hybrids between wild plants from 'Kushikino' and the cultivars had the Ogura-type mtDNA, whereas a part of those between 'Tomioka' and the cultivars showed a normal mtDNA (Table 1). These findings are consistent with our observation that the mtDNA of the plants in the 'Kushikino' population is fixed to the Ogura-type (YAMAGISHI and TERACHI 1994), whereas both the Ogura- and normal-type were present in the 'Tomioka' population. The presence of the two mtDNA types in the hybrid plants may reflect the variations in the original wild radish population.

All the hybrids using wild plants from 'Tomioka' as a female parent displayed a normal male fertility regardless of the mtDNA type and the pollen parent. On the other hand, half of the plants derived from crosses between 'Kushikino' and the three cultivars excluding 'Comet' (Table 1) did not develop normal anthers, and produced no fertile pollen. These male sterile plants were able to produce seeds by artificial crossing, indicating the presence of a normal female fertility. The segregation of the male sterility in the F₁ hybrids suggests that the 'Kushikino' population contains heterozygous plants for nuclear gene(s) related to male sterility. In this case, the male sterile segregants should be included in wild radish populations. This assumption is supported by the observation that male sterile plants can be found occasionally in wild radish populations, though the frequency is less than 5% (H. YAMAGUCHI, personal communication).

Male sterility of 'MS-Gensuke' was maintained by both the crosses with 'Uchiki-Gensuke' and 'Kangoshi-Tokinashi' as male parents. By contrast, all the four hybrid plants between

Table 1. Mitochondrial DNA type and pollen fertility of the hybrids between wild and cultivated radishes

Cross combination ¹⁾		Number of F ₁ hybrids	Type of mtDNA	Male fertility	
Female	Male			Sterile	Fertile
Kushikino	Uchiki-Gensuke	4	Ogura	2	2
Kushikino	Aokubi-Miyashige-Oonaga	4	Ogura	2	2
Kushikino	Kangoshi-Tokinashi	4	Ogura	2	2
Kushikino	Comet	4	Ogura	0	4
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Tomioka	Uchiki-Gensuke	4	Ogura	0	4
Tomioka	Aokubi-Miyashige-Oonaga	3	Normal	0	3
Tomioka	Kangoshi-Tokinashi	4	Ogura	0	4
Tomioka	Comet	2	Ogura	0	2
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MS-Gensuke	Uchiki-Gensuke	3	Ogura	3	0
MS-Gensuke	Kangoshi-Tokinashi	3	Ogura	3	0
MS-Gensuke	Comet	4	Ogura	0	4

¹⁾ Four plants were used as the female parent for each of the crosses, and the F₁ seeds obtained in a cross combination were mixed.

Fig. 1. Development of anthers in the hybrids of radish (In the flowers B and D normal development was observed as indicated by arrows.).

- A; 'Kushikino' × 'Uchiki-Gensuke' (Sterile)
 B; 'Kushikino' × 'Comet' (Fertile)
 C; 'MS-Gensuke' × 'Uchiki-Gensuke' (Sterile)
 D; 'MS-Gensuke' × 'Comet' (Fertile)

'MS-Gensuke' and 'Comet' showed a normal fertility. This means that 'Comet' has restorer gene(s) to the Ogura male sterile cytoplasm. Normal pollen fertility was also observed in all the F₁ plants derived from the cross between 'Kushikino' and 'Comet' (Table 1, Fig. 1). These findings suggest that the male sterility observed in the hybrids between 'Kushikino' and the cultivars is controlled by the same genetic system as that of the Ogura cytoplasmic male sterility, which indicates the possibility that the Ogura cytoplasm originated in the wild Japanese radish. To verify this assumption, progeny tests are being conducted. Besides the tests, further studies on the relationship with the cytoplasm of 'Kosena' (SAKAI and IMAMURA 1990), and the influence of the temperature on the sterility (NIEUWHOF 1990) would be necessary, for the strict determination of the inheritance mode of this male sterility.

Acknowledgements

We thank Drs. O. OHNISHI and M. YAMABE for kindly providing us with the seeds of wild and male sterile radishes. We also thank Ms. M. SHINOJIMA for her help in preparing the manuscript.

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ハマダイコンと栽培ダイコンの交雑による雄性不稔個体の出現

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アブラナ科作物で広く利用されているオグラ型雄性不稔性細胞質の起源を解明する手がかりを得ることを目的として、ハマダイコンと栽培ダイコンとの雑種について、ミトコンドリアDNAのタイプ並びに雄性不稔性の発現を調査した。

鹿児島県串木野市及び熊本県富岡町で収集されたハマダイコンを母本として、栽培品種（‘打木源助’、青首宮重大長、寒越し時無、コメット）との間に雑種を得て本実験に用いた。また対照としてオグラ型細胞質を持ち雄性不稔性を示す‘MS源助’を母本とする雑種を供した。雑種幼苗の葉より全DNAを抽出し、ミトコンドリアの*atp 6* 遺伝子上流におけるオグラ型特異的及び正常型特異的な塩基配列を標的として、それぞれPCRを行った。その後、各雑種個体の開花時に葯の発達を観察して雄性不稔性の判定を行った。

PCRの結果、串木野市のハマダイコンを母本とする雑種はすべてオグラ型のミトコンドリアDNAを有していたのに対して、富岡町のハマダイコンを母本とする雑種には正常型のミトコンドリアDNAを持つ個体が含まれていた（Table 1）。これは元のハマダイコンの集団における細胞質の分化を反映したものであると考えられた。富岡町のハマダイコンを用いた雑種は、すべて正常な葯の発達を示したのに対して、串木野市のハマダイコンに由来する雑種では‘打木源助’、青首宮重大長、寒越し時無’との組み合わせで半数の個体が不稔性を示した（Table 1）。これら不稔個体は栽培品種の授粉によって結実し、雌性器官は正常であることが示された。一方、‘MS源助’と‘コメット’との交配によって花粉稔性が回復することが認められたが、同様に串木野市のハマダイコンと‘コメット’との雑種においても全個体が可稔となった。（Table 1, Fig. 1）。

これらのことから、本実験で出現した雄性不稔性はオグラ型雄性不稔性と同一である可能性が示唆され、これよりオグラ型細胞質は我国に自生するハマダイコンに由来したものである可能性が考えられた。現在、更に後代検定を進めている。