Notes Plant Biotechnology, 14(2), 119-121(1997)

Analysis of the Mitochondrial and Chloroplast Genomes Associated with Stamenless-type Cytoplasmic Male-sterility in Tomato

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Received 20 August 1996; accepted 12 April 1997

Introduction of the cytoplasm of wild potato (*Solanum acaule*) to tomato by asymmetric cell-fusion created tomato lines exhibiting various cytoplasmic male-sterile (cms) phenotypes [1]. The most common cms lines (represented by MSA1) had morphologically normal flower structures but the pollen did not germinate (pollen-type cms) [1]. The physical mappig of the MSA1 mitochondrial genome showed that it consists of a complex mixture of the parental genomes with at least 11 molecular recombination events [2]. We here analyzed the mitochondrial and chloroplast genomes from another fusion product, RCMSA4, which exhibited abnormality in stamen development (stamenless-type cms) and compared them with those of MSA1.

RCMSA4 was recovered among regenerated plants from asymmetric cell-fusion between protoplasts of tomato (*Lycopersicon esculentum* var. *cerasiforme* cv. Red Cherry) treated with iodoacetamide and γ-irradiated protoplasts of wild potato (*Solanum acaule*) [1]. Figure 1 shows the morphological comparison of the wild type and RCMSA4 flowers. While a pistil is covered by five stamens in a wild type flower (**Fig. 1A**), a naked pistil extrudes in a RCMSA4 flower (**Fig. 1B**). Whereas the nuclear background of MSA1 is *L. esculentum* cv. Sekai-ishi, that of RCMSA4 is *L. esculentum* var. *cerasiforme* cv. Red Cherry. The different phenotypes are, however, unlikely to be due to the differences in the tomato cultivar used in protoplast fusion, since we observed stamenless plants among the fusion products with the Sekai-ichi background, and MSA1-type cms plants among fusion products with the Red Cherry background (data not shown).

Nuclear background of RCMSA4 was exchanged by repetitive back-crossing with commercially important cultivar MN. All resulting progenies lacked fully developed and functional stamens without segregation, but occasionally developed stamen-like structure aborted at various stages (**Fig. 1C**). Although the MN nuclear background caused this incomplete restoration of stamens, variable phenotypes may be due to physiological differences between flowers rather than genetic differences, since flowers of an identical plant often showed different phenotypes.

Introduction of alloplasmic cytoplasm often causes various phenotypic aberrations in reproductive organs as well as pollen abortion. In the genus *Nicotiana*, stamen and petal development is affected by various male-sterile cytoplasms [3-5]. Aborted stamen development in our RCMSA4 tomato is phenotypically similar to *N. tabacum* carrying *N. sauveolens* cyto-

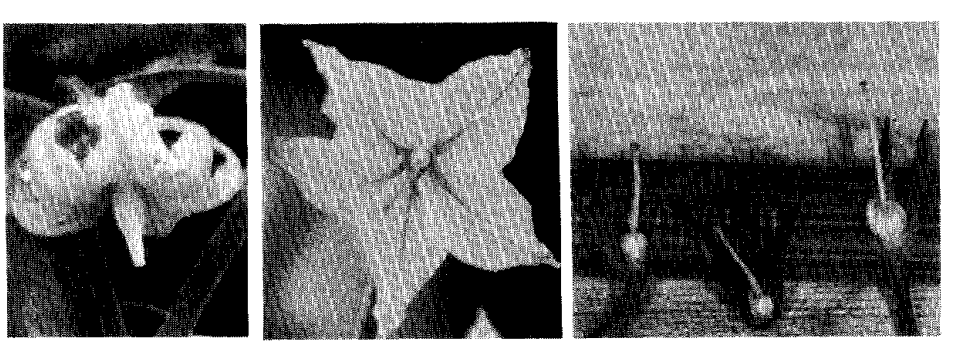

Fig. 1 A wild type flower of Red Cherry (A) and a stamenless flower of RCMSA 4 (B) and BC_2 progenies of RCMSA 4 pollinated with MN (C).
Sepals and petals were removed to show the morphology of stamen in (C).

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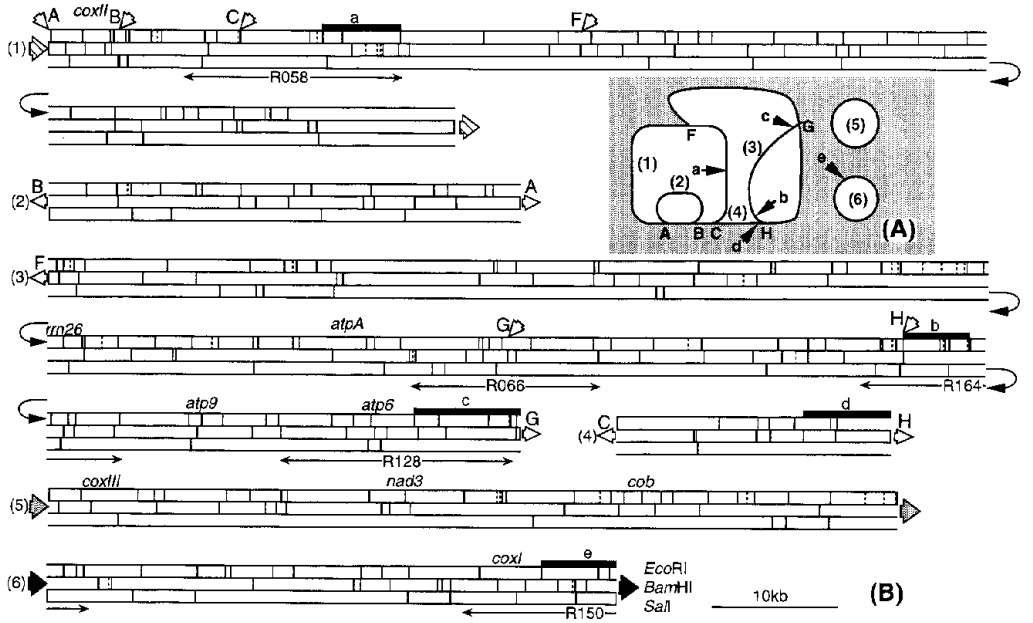

Fig. 2 Overall physical map of the RCMSA4 mitochondrial genome.
(A) Schematic representation of the positional relationship of the six maps. (B) Physical maps of the RCMSA4 mitochondrial genome. The entire genome is represented by six maps (1)-(6), which are depicted in linear forms. Maps 1, 5 and 6 are three separate circular maps, whereas ends of maps 2, 3 and 4 (A, B, C, F, G and H) link to each branching point in map 1 and 3. Labeled arrows show the position and direction of branching. Approximate positions of nine mitochondrial genes are indicated by their genetic symbols. Vertical dotted lines indicate fragments, alignment of which were not determined. Labeled bars (a-e) indicate RCMSA4-specific sequences. The locations of the phage clones used for probes are also indicated.

plasm. Cytoplasms of both alloplasmic cms lines may disturb a similar function that is important for, or sensitive in, normal floral development but not for vegetative growth.

Mitochondrial genome of RCMSA4 was analyzed by constructing the physical map (**Fig. 2**). It was represented by three circular (maps 1, 5 and 6) and three linear maps (maps 2, 3 and 4) to avoid large sequence duplications. Even if large direct repeats and inverted repeats were allowed, overall genome structure could not be depicted by a single circle (master circle) as that of MSA1 could not [2]. In the process of extending maps, two different genomic sequences sometimes converged into the same DNA fragments, thus creating branching points. The four branching points (A, B, C and F) were also found on the map of MSA1 mitochondrial DNA [2], whereas the two branching points, G and H, were unique in RCMSA4. The mitochondrial genome size of RCMSA4 was approximately 470 kb, which is slightly larger than that of MSA1 (450 kb).

Comparison of physical maps between PCMSA4 and MSA1 revealed that five sequences (a)-(e)(**Fig. 2B**) are specific to RCMSA4. Sequences (b), (c) and (d) were present at the junction sites of RCMSA4-specific branching points G and H. Southern analysis showed that the RCMSA4-specific sequences were originated

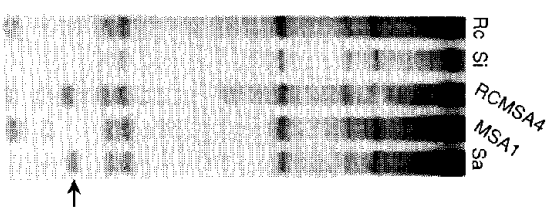

Fig. 3 Representative hybridization pattern of total DNA extracted from *L. esculentum* var. *cerasiform* cv. Red Cherry (Rc), *L. esculentum* cv. Sekai-ichi (Si), RCMSA4, MSA1 and *Solanum acaule* (Sa).
DNA digested with *Xba*I was hybridized with the cosmid clone covering the single-copy region of chloroplast DNA. Fragments showing RFLP are indicated by arrows.

from wild potato and were created by the insertion of extra sequences absent in MSA1, rather than by the different recombination (data not shown). The physical map of the RCMSA4 mitochondrial genome may guide to search for the specific DNA region responsible for the stamenless-type cms.

In *Brassica*, Southern analysis of the mitochondrial genome of fusion products showed that molecular recombination did not occur at random [6]. Our comparison of the genome structure between RCMSA4 and MSA1 agrees with this previous obser-

vation. Although the overall genome stucture was quite different between RCMSA4 and MSA1 due to the different recombinations and the insertion of five RCMSA4-specific sequences, the maps of both fusion products were similar to each other at many points.

We next analyzed the origin of RCMSA4 chloroplast genome. Five cosmid clones covering the whole tomato chloroplast sequence were selected from the chloroplast DNA library of Red Cherry and used as hybridization probes. Although chloroplast DNA sequences were well conserved between tomato and wild potato, some combinations of the restriction enzymes and probes were able to distinguish them (**Fig. 3**). All probes detected RFLP (Restriction fragment length polymorphisms) specific to wild potato (data not shown), indicating that the entire RCMSA4 chloroplast genome was derived from wild potato without recombination with the tomato genome. Similar analysis using MSA1 showed that the entire MSA1 chloroplast genome originated from tomato without recombination with the wild potato genome.

Since the plant growth was indistinguishable between RCMSA4 with other cms lines carrying the tomato chloroplast genome, the heterologous chloroplast genome was unlikely to impair the plastid function in vegetative cells. Nonetheless, the wild potato chloroplast genome might interfere with the development of reproductive organs, since it cosegregated with the abnormal developments of stamen and/or female reproductive organs among additional cell-fusion products between RCMSA4 and the fertile tomato (unpublished data).

Acknowledgments

We are grateful to Dr. Takashi Hashimoto of Nara Institute of Science and Technology for his critical reading of the manuscript.

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