

The mechanism of sex determination in sugar beet: nuclear-mitochondrial interaction alters the fate of pollen.

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Introduction: Sugar beet (*Beta vulgaris* L.) is commonly hermaphrodite, but the pollen sterile plants were discovered in 1940s. They are functionally female, and have been utilized in the production of F1 hybrid. In most allogamous plants, F1 hybrid by crossing two genetically different strains shows growth vigor, so it has been exploited to plant breeding. For commercial production of F1 hybrid seeds, female has been used as seed parent, because they cannot put self-crossed seed. Almost all sugar beet lines are F1 hybrid lines, so it is essential to elucidate the mechanism of sex determination in sugar beet.

The femaleness is determined by nuclear-mitochondrial interaction. Pollen sterility is induced by mitochondrial gene, which repressed by the nuclear *restorer-of-fertility* (*Rf*) gene. The molecular entity of them had been explored. Comparing the nucleotide sequence of pollen sterility-inducing and non-sterilizing mitochondrial genome, some genes unique to sterility-inducing mitochondria were discovered. Among them, only the product of *preSatp6* was detected; therefore it appears that *preSatp6* is associated to pollen sterility. On the other hand, *Oma1*-like gene was cloned as the nuclear *Rf* gene by positional cloning. However, the molecular mechanism of the interaction between *preSatp6* and *Oma1*-like gene has not been investigated.

Aim: We attempt to describe how *Rf* gene interacts and represses *preSatp6* on molecular level.

Result and Discussion: Yeast OMA1 has the chaperone-like activity, so it is possible that RF protein bind to preSATP6. Confirming this possibility, the protein complex including preSATP6 was collected by co-immunoprecipitation assay using anti-preSATP6 antibody. This collection included RF protein, so it is suggested that RF protein binds to preSATP6. Next, we thought that RF-preSATP6 interaction may result in conformational alternation of preSATP6 complex. To see the effect of RF on preSATP6, signal bands of preSATP6 in *Rf*-expressing and non-expressing anthers were compared by immunoblotting combined with blue-native polyacrylamide gel electrophoresis. The state of preSATP6 protein complex was altered in *Rf*-expressing anther, and the one of preSATP6 complex is likely to include RF protein.

In sugar beet, *preSatp6* appears to cause disrupting pollen development, while *Rf* restores pollen fertility by repressing *preSatp6* through post-translational way. preSATP6 protein *per se* may not be harmful to pollen development, but preSATP6 complex may be.

Key Words: pollen sterility, restorer-of-fertility, plant mitochondria, protein-protein interaction

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