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New molecular approaches to develop conditional male-sterile plants in *Arabidopsis* and Chinese cabbage

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Objectives

Our aim in this research is to develop new approaches using a conditional male-sterility for the F1 hybrid seed production in Chinese cabbage. Now, we are constructing vectors required for this goal.

Materials and Methods

1. Materials

Bacterial strains: *Escherichia coli* JM109, *Agrobacterium tumefaciens* LBA4404

Plant materials: *Arabidopsis thaliana*, *Brassica campestris*

Anther specific promoters: *gex2* (1.8kb), *msp2* (1.0kb), *lats2* (0.5kb)

Target genes: allene oxide synthase gene (AOS) of *Arabidopsis* for jasmonic acid biosynthesis

2. Methods

Most of experiments were performed by general methods for gene cloning. Binary vectors for RNAi approach have been constructed by the gateway cloning technology (Invitrogen).

Results and Discussion

Jasmonic acid(JA) is a signaling molecule essential for anther development and pollen fertility in *Arabidopsis*. To induce a conditional male-sterility in plant, we have designed anther not to synthesize JA by inhibiting anther-specifically AOS important for JA biosynthesis. The three binary vectors, pK7gex2, pK7msp2, and pK7lat52 with promoter region of anther-specific genes of *Arabidopsis*, *gex2*, *msp2*, and *lat52* have constructed respectively. About 600bp fragment of AOS was amplified from genomic DNA of *Arabidopsis* and cloned into pK7gex2, pK7msp2, and pK7lat52. The final binary vectors were named pK7gex2AOS, pK7msp2AOS, and pK7lat52AOS, which were carrying anther-specific promoter::AOS RNAi structure. The binary vectors will be introduced into *Arabidopsis* and Chinese cabbage plants. We expect that the transformed plants with the RNAi molecules have a defect only in anther development or pollen fertility without other abnormal growth. In addition, we expect that when JA is treated to the male-sterile plants in flowering stage, the fertility of the plants is recovered.

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