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Cloning and analysis of anther specific cDNAs and their promoters in the apple

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In this study, we performed development of the anther-specific promoters and cDNAs which are useful for generating male sterile plants. Anther specific cDNA (*MdASG1*) in the apple (*Malus* x *domestica*) was isolated by differential display PCR analysis. Analysis of *In situ* hybridization indicated that the *MdASG1* transcript was preferentially expressed in tapetum and in the junction of pollen sac and filament in the anther. In addition, we isolated two cDNAs (*MdASG2* and *MdASG3*), which have high sequence similarity to the *MdASG1*. The putative protein products of the *MdASGs* (*MdASG1,2,3*) were 7~8 kD novel arabinogalactan polypeptides that expressed in pollen specific manner. Transgenic tobacco, expressed *MdASGs* anti-sense mRNA, showed an abnormal phenotype in anther. *MdASGs* suppression caused retardation of pollen development and reduction of seed settings compared with those of selfing wild type plants. In order to investigate anther specific promoters, we obtained the 5' upstream sequences of *MdASGs* from genomic DNA and characterized their promoter regions. We also made *MdASG2* promoter-GUS construct and introduced to tobacco. Fluorometric and histochemical GUS analysis showed that a *MdASG2* promoter-GUS fusion product exhibited temporally and spatially specific expression in early stage of microsporogenesis. The transgenic tobacco that contained an anti-sense *MdASG2* gene under the *MdASG2* promoter showed a novel phenotype that include a defect of pollen development. For further analysis, we obtained hybrid seeds of male sterilized lines, which have a heritable and stable trait, by breeding male sterilized transgenic tobacco pollinated with wild type pollen.

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