

Search for Megasporogenesis Genes in Alfalfa

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Knowledge on the regulation of female meiosis is still very limited, but it can have a significant impact on reproduction control and seed production. Several sporophytic mutations influencing both male and female meiosis have been described, but there are only two reports of mutations determining the block of female meiosis only (Siddiqi *et al.* 2000, *Development* 127: 197-207; Motamayor *et al.* 2000, *Sex. Plant Reprod.* 12: 209-218).

We are studying a mutation causing female-specific megasporogenesis abnormalities and female sterility in alfalfa, involving an arrest of sporogenesis at early prophase associated with ectopic, massive callose deposition within the nucellus (Rosellini *et al.* 2003, *Sexual Plant Reprod.* 15:271-279).

With the purpose of isolating genes involved in the megasporogenesis process, the cDNA-AFLP technique was employed to isolate genes differentially expressed between wild type and female-sterile full sib alfalfa plants. Ninety six ESTs were generated, and most of them published (GenBank from CB165074 to CB165159). BLAST analysis have revealed similarities with genes involved in the cell cycle, development and callose metabolism.

Four clones were selected to proceed with further studies: CB165076 similar to *Arabidopsis thaliana* eukaryotic initiation translation factor eIF4G III; CB165091 similar to a soybean 1,3 beta-glucanase; CB165105 similar to the *A. thaliana* transcription factor SCARECROW gene regulator; and CB165125, similar to an *A. thaliana* MAPKKK.

Full length mRNA sequences were obtained using the Rapid Amplification of cDNA Ends (RACE) technique. RT-PCR was performed to confirm differential expression during flower development using the 18s rRNA as control gene. *In situ* hybridization experiments are carried out to spatially characterize the expression of the selected genes.