A Molecular Study of Alfalfa Megasporogenesis

D. Rosellini
Università di Perugia, Italy

Stefano Capomaccio
Università di Perugia, Italy

F. Veronesi
Università di Perugia, Italy

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D. Rosellini, S. Capomaccio and F. Veronesi
Department of Plant Biology and Agro-environmental Biotechnology, Borgo XX Giugno, 74 - 06121 Perugia (Italy). Email: roselli@unipg.it

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Introduction Our ability to control plant reproduction impacts on both seed production and plant breeding. A female sterility mutation was previously described (Rosellini *et al*., 1998; 2003) revealing a female-specific arrest of sporogenesis associated with ectopic, massive callose deposition within the nucellus. The goal of this study is to isolate and characterize genes involved in megasporogenesis and female sterility in alfalfa.

Materials and methods In a 50-plant F_1 alfalfa population segregating for female sterility, ten plants were selected, 5 with sterility higher than 97% and 5 with sterility lower than 5%. Flower buds were excised from each plant at five flower development stages; leaves were also sampled as a control. cDNA was synthesized from total RNA of each sample; two cDNA bulks were formed using the same amounts of cDNA from each of the sterile (sterile bulk) and fertile (fertile bulk) plants and cDNA-AFLP analysis was performed. Full-length cDNAs corresponding to four selected differentially expressed transcripts were isolated by the RACE technique and sequenced. RT-PCR was performed for these four genes to confirm differential expression.

Results 96 Expressed Sequence Tags (ESTs) differentially expressed between sterile and fertile bulks were generated, and most of them published (GenBank from CB165074 to CB165159). Similarities with genes involved in the cell cycle, development and callose metabolism were found. Four were chosen for further studies: CB165076 similar to *A. thaliana* eukaryotic initiation translation factor *eIF4G III*; CB165091 similar to a soybean flower *beta 1,3-glucanase*; CB165125 similar to an *A. thaliana MAPKKK*; CB165105 similar to the *A. thaliana* transcription factor *SCARECROW* gene regulator. RT-PCR confirmed the differential expression between sterile and fertile plants for three of these genes, and flower specificity for two of them (Figure 1). Different alleles of CB165076 are probably revealed by RT-PCR performed with different primer pairs. The full length cDNA sequences (Table 1) revealed that the *eIF4G III*-like gene is much shorter than the similar published genes (not shown) and misses the MIF4G domain (RNA, DNA, protein binding).

Table 1 Features of the isolated cDNAs

Clone	Similar to	cDNA nt	CDS nt
CB165076	At3g60240	4084	3468
CB165091	AAC04713	2103	1692
CB165125	CAB87658	1379	1050
CB165105	At5g66670	2320	1656

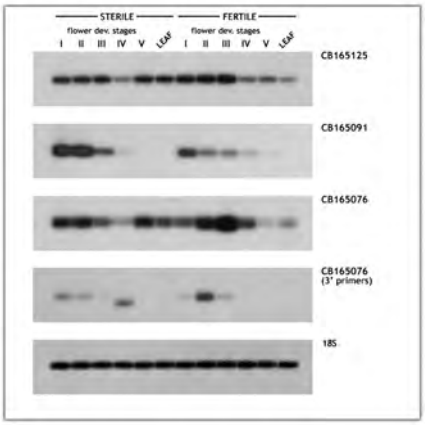

Figure 1 RT-PCR experiments on three differentially expressed genes

Conclusions Among the 96 ESTs, similarities for genes involved in the cell cycle, development and callose metabolism were found. Full-length cDNAs were cloned for four alfalfa genes whose similarity with published sequences suggests a possible involvement in the sterility trait. Our work continues with cloning and sequencing the genomic regions of these genes and with Southern and *in situ* hybridization experiments to assess gene copy number and expression patterns. This research was funded by the Italian Ministry of University and Scientific and Technological Research (project: Isolation and mapping of genes affecting sporogenesis and gametogenesis in *Medicago* spp) and is part of S. Capomaccio's *Dottorato di Ricerca* research activity.

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