SUPPLEMENTARY MATERIALS AND METHODS

Real-time RT-PCR
Expression levels of seven genes were analyzed using real-time, quantitative PCR as a validation of microarray results. Reverse transcription was performed with the M-MLV Reverse Transcriptase (Promega), using aliquots of total RNA extracted for microarray analysis. The cDNA samples were diluted to 20ng/μl. Gene-specific primers designed by using PRIMEREXPRESS software (Applied Biosystems) are shown in Supplemental Table S2. All real-time PCR reactions were performed using the ABI 7700 sequence detection system (Perkin-Elmer Applied Biosystems) and the amplifications were done using the SYBR Green PCR Master Mix (Applied Biosystems). The thermal cycling conditions were composed of 50°C for 2 min followed by an initial denaturation step at 95°C for 10 min, 45 cycles at 95°C for 30s, 60°C for 30s and 72°C for 30s. The experiments were carried out in duplicate for each data point. The relative quantification in gene expression was determined using the 2^ΔΔCt method (Livak and Schmittgen 2001). Using this method, we obtained the fold changes in gene expression normalized to an internal control gene, and relative to one line (calibrator). The contig3116_at, coding for a mitochondrial ATP synthase delta chain, was used as an internal control to normalize all data and the line DH46 was chosen as the calibrator.

Semi-quantitative RT-PCR
Expression analyses of seven genes in four different developmental stages were conducted by semi-quantitative RT-PCR. Total RNA was extracted from anthers as explained for microarray analysis. Reverse transcription was performed with the M-MLV Reverse Transcriptase (Promega) and the resulting cDNA was diluted to 20ng/μl. Gene-specific primer pairs were designed using PRIMEREXPRESS software (Applied Biosystems) and are shown in Supplemental Table S2. PCR reactions were optimized to 94°C for 3 min, 33 amplification cycles at 94°C for 1 min, the appropriate annealing temperature (Table S2) for 1 min, 72°C for 1 min, and a final extension of 10 min at 72°C. Amplified products were resolved on 2% agarose gels and visualized by ethidium bromide staining.
DAPI staining
Fresh anthers were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C overnight. Microspores isolated from anther tissue were stained in 1ng/ml DAPI (4’,6-diamino-2-phenylindole) aqueous solution containing 1% Triton X-100 (Vergne et al. 1987). Microspores were observed under a fluorescence microscope (Nikon Eclipse T300) using UV light.

Semithin sections
Anthers fixed in 4% paraformaldehyde were dehydrated in ethanol series, after which they were immerse in “Infiltration Solution” (Historesin, Leica) overnight at 4°C and embedded subsequently in “Embedding Medium” (Historesin, Leica). Semithin sections (5μm) were stained with toluidine blue 0.05% and observed under bright light in a microscope Nikon Eclipse T300.

REFERENCES