SETTING-UP OF LASER MICRODISSECTION FOR CAPTURING PLANT MALE MEIOCYTES

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LCM, meiosis

So far, plant meiotic genes have been isolated through comparative analysis with model organisms like yeast and drosophila or, alternatively, by forward genetics approach in Arabidopsis thaliana mutants. We are setting up a methodology which combines the single cell technology based on Laser Capture Microdissection (LCM) and the microarrays for a large-scale analysis of gene expression to elucidate the meiotic process.

Arabidopsis thaliana ecotype Col-0 was grown at controlled conditions and floral buds were processed by LCM to collect meiotic and somatic cells. In order to preserve the morphology of floral structure the tissues were fixed in Farmer’s solution and infiltrated in 30% sucrose to be criosectioned. Meiotic stages were recognized on few sections of each floral bud stained with 4-6-diamidino-2-phenylindole (DAPI) and the cell capture was performed on unstained sections of the corresponding sample. LCM Arcturus Pix Cell II system was set up by evaluating parameters of “capture efficiency” to isolate somatic cells from sepals and meiocytes from anthers and the number of captured cells to be used for RNA extraction. RNA yield and quality was evaluated according to nanodrop ND-1000 and Agilent Technologies, respectively, and RNA specificity was verified through RT-PCR. LCM system parameters are the same for both somatic and meiotic cells. In particular, power range was 90-100 mW, duration 1.0-1.5 ms and spotsize 7,5 µm. The number of somatic cells is about ten thousand to recover 10-20 ng/µl RNA while the meiocyte number is about 4000 to obtain 7-15 ng/µl RNA. The ratio 28S/18S indicating RNA quality is fair. To verify the absence of somatic cell contamination of meiocyte population and the presence of meiocytes at early meiotic stages, when polyA’RNA is abundant, RT-PCR amplification analysis was carried out with a tapetal specific marker (ATA7) and with two meiotic genes expressed at prophase I stage (SWI1 and DMC1). The following step is to obtain RNA amplification to allow the microarray analysis.