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Poster Abstract – D.45

TOWARDS THE USE OF LASER CAPTURE MICRODISSECTION FOR INVESTIGATING PLANT MEIOSIS

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The molecular mechanisms underlying plant meiosis are widely unknown and the number of plant meiotic genes, so far identified, is very restricted. The methodologies based on differential gene expression analyzed bulk samples containing a mixture of heterogeneous cells at different developmental stages. As a consequence, these methodologies were not ideal for meiosis investigation in plants. A methodology which combines the single cell technology and the analysis of gene expression using microarrays could unravel the molecular understanding of meiosis at genomic level. LCM is a powerful technique by which individual targeted cells can be harvested from heterogeneous tissues while they are viewed under the microscope, by tacking selected cells to an adhesive film with a laser beam. So far, LCM has been widely applied on animal tissues, and few studies have shown that LCM can be a powerful tool in plants, as well. However, meiotic cells have never been isolated by LCM. Recently, our research group started setting up Laser Capture Microdissection (LCM) in *Arabidopsis thaliana* with the aim of harvesting premeiotic and meiotic cells to obtain RNA for expression profiling.

Ecotype Col-0 of *Arabidopsis thaliana* was grown at controlled conditions and floral buds were collected and processed according to histology techniques for paraffin embedding or cryosectioning. The two methods were compared for section integrity that is crucial for morphological recognition of the meiotic stage. Paraffin sectioning preserves the tissue morphology better than cryosectioning but it is known that RNA recovering is generally worse. An important step is the choice of fixative mixture that in our case was Carnoy (ethanol-acetic acid 3:1) with non cross-linking property. In cryosectioning, 10% sucrose solution in PBS-DEPC was used as a cryoprotectant for infiltration of floral buds before chilling. The staining is another crucial step to identify the meiotic stage but not all nuclear staining substances allow to recover RNA. Different staining protocols were tested including Mayers' and Harris' hematoxylin, methyl green, acetic carmine and cresyl violet acetate. According to preliminary experiments, LCM parameters for capturing meiocytes have been set.