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TRANSCRIPTOMIC ANALYSIS OF OVULE-SPECIFIC CELL LINEAGES TO IDENTIFY GENES RELATED TO APOSPOROUS APOMIXIS IN *HYPERICUM PERFORATUM* L.

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Apomixis defines a plant reproductive strategy that, unlike sexual reproduction, permits the inheritance of the maternal genome without genetic recombination and syngamy. Earliest phenotypical symptoms of the aposporous developmental program in ovules are the avoidance of meiosis (*i.e.*, apomeiosis) and the differentiation of functional unreduced embryo sacs from somatic cells of the nucellus (*i.e.*, aposporous initials). Apospory deviates from sexuality as, in this latter case, the commitment to develop an embryo sac is strictly restricted to the reduced functional megaspore (FM) and failure of the meiotic program is typically not accompanied by the initiation of embryo sac development from cell lineages of the ovule other than the FM.

Our research goal is a better understanding on gene expression changes accompanying the onset of aposporous apomixis in the ovule of the model species *H. perforatum* L. To this purpose, gene expression analyses were performed by adopting the RNA-seq technology on Laser-Capture Microdissected (LCM) ovule cells collected from sexual and apomictic genotypes at pre-meiotic developmental stages. We identified 402 differentially expressed genes (DEGs) (Bonferroni p-value ≤ 0.05) between ovules belonging to sexual and apomictic genotypes. Among these, 97 transcripts were only found in apomictic libraries, suggesting apomictic-specific expression. At the same time, 25 transcripts were only detected in sexual libraries. Differential expression was validated by Real-Time qPCR and in-situ hybridization assay. Among identified DEGs, we found several RNAs whose products are related to biological processes modulated in other aposporous apomictic model species. Ontological annotation revealed an enrichment of the following biological processes in apomictic ovules: RNA binding, RNA splicing and RNA-directed DNA polymerase activity, this latter being associated to putative non-LTR retroelements. The massive expression of TEs in apomictic ovules suggested that DNA methylation is compromised in these cells. To address this question, we investigated the promoter and gene body DNA methylation level of a subset of DEGs by chop-PCR assays. Gene body methylation level of DEGs annotated as putative non-LTR retroelements supports the idea that transcriptomic changes for these genes might be epigenetically controlled. Furthermore, several genes involved in auxin and cytokinin (CK) homeostasis and signalling were found differentially expressed, implying that apomictic ovules might be subjected to alternative hormonal interplays. This let us hypothesise that hormonal response and DNA methylation might be connected to the transcriptional changes observed in apomictic ovules. To address this question, gene expression and promoter methylation studies were performed on flowers treated with synthetic CK and its antagonist PI-55. Gene expression and DNA methylation data will be presented and critically discussed in the frame of ovule and gamete development. Overall, our data suggest that phenotypic expression of early events of aposporous apomixis in *H. perforatum* is

concomitant with the modulation of key genes involved in hormonal homeostasis, DNA methylation and cell cycle progression.