

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

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The medicinal plant St. John's wort (*Hypericum perforatum* L.) is an attractive model system for the study of aposporous apomixis. The key biological features of apomixis in this species are the by-passing of meiosis, the differentiation of aposporous initials into embryo sacs containing unreduced egg cells, their autonomous development in functional embryos without fertilization, and the formation of viable endosperm either via fertilization-independent means or following fertilization with a sperm nucleus. The aim of this research is to define gene expression changes occurring in the nucellar cell types of the ovules primarily involved in the differentiation of the megaspore mother cells and aposporous initials. To this end, a laser-capture microdissection approach was adopted in combination with the RNA-seq technology in order to restrict the frame of our investigations to a specific portion of the ovule, *i.e.* the nucellus, at developmental stages preceding the differentiation of aposporous initials. Overall, our gene expression analysis identified 270 and 81 unigenes that were found significantly up- and down-regulated between ovules collected from sexual and apomictic accessions. Ontological annotation of differentially expressed genes indicated that genes up-regulated in apomictic ovules were significantly enriched in ontological terms related to the RNA-directed DNA polymerase activity and the RNA binding. Among these genes, several actors of the RdDM pathway were found, suggesting that the phenotypic expression of early events of aposporous apomixis is associated to changes in *de novo* DNA methylation mediated by small RNAs. Furthermore, as deregulation of single components of the sexual developmental pathway is believed to be a trigger of the apomictic reproductive program, genes involved in sporogenesis, gametogenesis and response to hormonal stimuli were annotated and investigated in great detail. The expression analysis of candidate genes was performed not only by Real-Time qPCR but also by ISH assays in order to verify the temporal and spatial expression patterns of selected transcripts in the ovule. Finally, the activity of specific genes in relation to embryo sac and/or embryo formation was investigated by using *A. thaliana* knock-out lines. Overall, our data suggest that phenotypic expression of aposporous apomixis is concomitant with the modulation of key genes involved in the sexual reproductive pathway, hormones and other actors likely playing a crucial role in the RNA-directed DNA methylation pathway.