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OVULE SPECIFIC TRANSCRIPTOMAL EVOLUTION IN THE *RANUNCULUS AURICOMUS* COMPLEX

Pellino M[1], **Hojsgaard** D[2], **Hörandl** E[2], **Schmutzer** T[1], **Vogel** H[3], **Scholtz** U[1], **Sharbel** T[1]

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Apomixis, clonal reproduction through seed, is a puzzling process that has focused the scientific community for many years. Evolved independently in many taxa from sexual ancestors, it remains an unresolved process that has interesting evolutionary implications as well as great economical and agricultural potentials. One hypothesis for the induction of apomixis from sexual reproduction is heterochronic gene expression (i.e changes in relative expression through time) as a consequence of hybridization. Here we took advantage of the *Ranunculus auricomus* complex and its well-studied taxonomy to select 2 sexual species and their intermediate apomictic hybrid for a targeted gene expression study. RNA-seq data was used to custom design a 1.3K spot microarray that was used to hybridize cDNA from 4 ovule developmental stages in both apomictic and sexual genotypes. In total 28 arrays were run, and their analyses allowed us to identify ovule and stage specific gene expression patterns. Moreover, using high quality single nucleotide (SNP) polymorphisms mined from each species we explored the evolutionary consequences of asexuality (i.e Mullet's ratchet) and analyzed the genomic effects of hybridity, polyploidy and allelic divergence.

CONSTRUCTION OF FLORAL TRANSCRIPTOME AND MIRNA DATABASES FOR APOMICTIC AND SEXUAL GENOTYPES OF *ERAGROSTIS CURVULA*

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Eragrostis curvula is a perennial grass native to Southern Africa and widely distributed in semiarid regions of Argentina. Some cultivars are polyploids (from 4x to 8x) and reproduce by diplosporous apomixis, mode of reproduction strongly affected by ploidy. Diploids (2n = 2x = 20) are always sexual and very infrequent. Here we report the construction of floral transcriptome and miRNA databases for apomictic and sexual genotypes of this grass as important tools to study the complex nature of this intriguing reproductive mode. Total RNA was extracted from flowers at different developmental stages from the tetraploid genotypes Tanganyika USDA (apomictic) and OTA USDA (sexual). Two samples from each genotype were collected (two different plants, biological replicas constituted by a mix of different developmental stages) and the same RNA was divided in order to sequence the transcripts and the miRNAs. Sequencing was carried out at INDEAR (Instituto de Agrobiotecnología de Rosario, Rosario, Argentina). Each library (sexual or apomictic) by using the 454 GS FLX+ Roche method, according to the protocol provided by the manufacturer. miRNA were sequenced using the Illumina platform at GenXpro (Germany). The total number of reads from the transcriptome was 2,617,197, with a total number of 952,693,285 bp and an average read length of 364.01 bp. Transcripts de novo assembly (Newbler software package) gave a total of 49,568 contigs (~80,000,000 bp) and 133,782 singletons (~40,000,000 bp). Annotation was done by using the software BLAST2GO, which assigns Gene Ontology terms, being the most represented categories biological, biosynthetic and metabolic processes, reproduction, response to stress, small molecule metabolic process and transport. From these genes, 27, 806 were shared for both, apomictic and sexual genotypes and 3,384 and 6, 366 transcripts were detected only in the sexual and apomictic genotype, respectively. From these, ~45% could not be annotated (~5,000 genes). The use of the software MEGAN allowed us to detect approximately 5000 genes that are not represented in other plant species and that are excellent candidates to analyze. Relative to miRNA, 1,670,483 and 2,274,914 reads were detected in the sexual and apomictic genotype respectively. The role of these elements in the reproductive mode is being investigated.