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RNA-SEQ OF ALFALFA NEOTETRAPLOIDS

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Sexual polyploidization is one of the mechanisms of evolution in plants, with implications in domestication and agriculture. We used RNA-seq to investigate the gene expression changes triggered by chromosome doubling in full-sib diploid ($2n=2x=16$) and tetraploid ($2n=4x=32$) plants obtained by crossing two diploid *Medicago sativa* plants that produce n and $2n$ eggs or pollen, due to mutations in the meiotic pathway. Comparing $4x$ and $2x$ progeny plants from the same parents allows us to dissect the transcriptional effects of polyploidization from those of hybridization. Leaves at the vegetative stage were sampled from three biological reps (clonal propagules) of three $2x$ and three $4x$ progeny plants, and from the parental $2x$ plants (24 samples in total). Total RNA was extracted and used for RNA-seq. Sequencing (Novaseq6000 $2x150bp$, 30 millions of fragments per sample) and de novo transcriptome assembly were performed by Sequentia Biotech. Raw fastq files were cleaned and trimmed with Cutadapt, mapped with STAR to a non redundant custom reference genome obtained by Chen et al. (Nat Commun 11, 2494, 2020) Reads on features (protein coding genes) were counted with FeatureCounts. Poorly expressed genes were filtered out and the final matrix composed of 54433 entries per 24 samples was fed to DESeq2 to evaluate differential transcription levels. Genes were considered differentially expressed with $\text{LogFC}>|1|$ and $\text{FDR}<0.05$. Gene Ontology analysis was performed using the GOEnrichment algorithm in a Galaxy instance.

Clustering of transcription levels closely reflected Ploidy and Genotypes for the entire experiment and for all comparisons. A total of 1726 genes differed between parental mean and $2x$ progeny (hybridization sensitive), 1453

between parental mean and 4x progeny (hybridization + polyploidization sensitive), whereas 1571 genes differed between 2x and 4x progenies (polyploidization sensitive). Of the latter, less than half (717) were affected by ploidy change only, and not by hybridization. Interestingly, Gene Ontology enrichment analysis of the ploidy-sensitive genes revealed that the biological processes terms "cell cycle", "microtubule based-processes", "response to stress", "defence responses", "interspecies interaction", were among the most significantly enriched for genes that were upregulated in 4x progenies. This is consistent with the fact that stress tolerance is often enhanced by polyploidization, and this will be assessed in our materials. Further studies will also investigate the role of smRNAs in protein-coding gene transcription as affected by ploidy change.