RNAseq-based analysis of alfalfa transcriptome in response to salt stress.

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Salinity is one of the major abiotic stresses that affect alfalfa productivity and increased salt tolerance in alfalfa has a great economic potential. Currently, only a handful of high- throughputs sequencing experiments have been performed for transcriptome analysis and identification of stress-responsive genes in alfalfa. Here we report results of Illumina RNA-sequencing in two alfalfa genotypes contrasting in salt tolerance. A total of 184,596,972 and 183,022,614 short reads (50 bp) were generated from cDNA libraries originated from AZ-88NDC (susceptible check) and AZ-GERM SALT-II (salt-tolerant at the germination stage) germplasms, respectively. Differential expression of alfalfa genes was assessed by using *de novo* strategy or by mapping reads to a reference genome of *Medicago truncatula. De novo* transcriptomes were assembled using Velvet and Oases software packages. Preliminary bioinformatics analysis showed that in the salt tolerant line 1,350 genes significantly (False Discovery Rate <0.01) changed their expression level (more than 2 fold) in response to 150mM salt. Among them were 650 with non-redundant IDs (blastx e-value cut-off 1e-10). Out of the latter genes, 7 are known in alfalfa, 558 in *M. truncatula*, 55 in *G. max*, 6 in *V. vinifera*, 4 in *P. trichocarpa* and 20 in other species, including *Arabidopsis*. In the salt sensitive line 743 genes were differentially expressed. Among 408 genes with non-redundant IDs (blastx 1e-10), 3 are known in alfalfa, 356 in *M. truncatula,* 40 in *G. max*, 1 in *V. vinifera* and other 8 in different species, including *Arabidopsis*. When *M. truncatula* (Mt3.5 data release) was used as a reference for transcriptome assembly, 1,179 genes in the salt tolerant line and 846 genes in the salt sensitive line significantly (more than 2 fold, FDR <0.05) changed their expression level in response to salt stress. The linear correlation coefficient between differentially expressed genes found by two different methods was $r>0.9$. Forty one and thirty six transcription factors (TF) changed their expression level under salt stress conditions in the salt-tolerant line AZ-GERM SALT-II and the susceptible line AZ-88NDC, respectively when reads were mapped onto the *de novo* assembled contigs. Forty two TF were found to be differentially expressed in each of the lines when *M. truncatula* was used as a reference genome for transcriptome assembly. Differentially expressed genes were annotated and assigned to known functional groups, biological processes and regulatory networks using *Arabidopsis* gene ontology. Overrepresented categories included ion binding (GO:0043167), antioxidant activity (GO:0016209), integral to membrane (GO:0016021), carbohydrate metabolism (GO:0005975) and other functional groups that are known to be involved in stress regulatory pathways. These data will be used to elucidate the role of genes associated with salt tolerance and to identify genetic markers that are linked to adaptation to salt stress.