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RNA-Seq Analysis of the Wild Barley (H. spontaneum) Leaf Transcriptome under Salt Stress

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Abstract : Wild salt-tolerant barley (Hordeum spontaneum) is the ancestor of cultivated barley (Hordeum vulgare or H. vulgare). Although the cultivated barley genome is well studied, little is known about genome structure and function of its wild ancestor. In the present study, RNA-Seq analysis was performed on young leaves of wild barley treated with salt (500 mM NaCl) at four different time intervals. Transcriptome sequencing yielded 103 to 115 million reads for all replicates of each treatment, corresponding to over 10 billion nucleotides per sample. Of the total reads, between 74.8 and 80.3% could be mapped and 77.4 to 81.7% of the transcripts were found in the H. vulgare unigene database (unigene-mapped). The unmapped wild barley reads for all treatments and replicates were assembled de novo and the resulting contigs were used as a new reference genome. This resultedin94.3 to 95.3%oftheunmapped reads mapping to the new reference. The number of differentially expressed transcripts was 9277, 3861 of which were uni gene-mapped. The annotated unigene- and de novo-mapped transcripts (5100) were utilized to generate expression clusters across time of salt stress treatment. Two-dimensional hierarchical clustering classified differential expression profiles into nine expression clusters, four of which were selected for further analysis. Differentially expressed transcripts were assigned to the main functional categories. The most important groups were ‘response to external stimulus’ and ‘electron-carrier activity’. Highly expressed transcripts are involved in several biological processes, including electron transport and exchanger mechanisms, flavonoid biosynthesis, reactive oxygen species (ROS) scavenging, ethylene production, signaling network and protein refolding. The comparisons demonstrated that mRNA-Seq is an efficient method for the analysis of differentially expressed genes and biological processes under salt stress.

Keywords : electron transport, flavonoid biosynthesis, reactive oxygen species, rnaseq
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