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De Novo Transcriptome Sequencing of *Ramonda serbica*: Identification of the Candidate Genes Involved in the Desiccation Tolerance

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Abstract

*Ramonda serbica* Panc. is a resurrection plant that can survive a long period of severe dehydration-desiccation. Desiccation induces cellular membrane integrity loss, protein aggregation, and denaturation, as well as accelerated generation of reactive oxygen species. However, *R. serbica* can fully recover its metabolic functions already one day upon watering [1]. The aim of our study was to obtain more insight into the desiccation tolerance mechanisms by differential *de novo* transcriptomics of hydrated (HL) and desiccated leaves (DL).

For *R. serbica* transcriptome construction, the total high-quality RNA from mixed samples of five biological replicates of HL and of DL separately, was extracted according to our previously optimised protocol [2]. Highly purified cDNA libraries were sequenced on an Illumina Hi-Seq platform. The ambiguous nucleotides, adapter sequences, and low-quality sequences were trimmed, and the quality of the reads was checked before and after the trimming. In total, 39608813 (with Q30=94%) and 37482969 (with Q30=94.1%) clean reads were obtained in HL and DL, respectively, and used to perform transcriptome assembly by Trinity software. After removing the redundancy, 189456 transcripts with 189003 unigenes were obtained (32.6% with the length between 500-1kbp).

Comparative analysis revealed that a large portion of *R. serbica* sequences (49.1%) exhibited high homology (according to obtained blast hits, e-value = 1e-5) with sequences found in the genome of another resurrection plant *Boea hygrometrica*. Furthermore, among the obtained unigenes (merged data for HL and DL), 64.6% and 42.3% were annotated by NCBI non-redundant protein and nucleotide sequences database (db), 23% by PFAM db, 22.5% by Clusters of Orthologous Groups of proteins db, 48.02% by Swiss-Prot db, 23% by KEGG db and 13.73 by Gene Ontology db. According to Blast2go analysis, the majority of annotated genes of *R. serbica* were associated with translation, ribosomal structure, posttranslational modifications, protein turnover, signalling pathways and cytoskeleton and encoded chaperonins and late embryogenesis abundant (LEA) proteins.

Aiming to provide a list of candidates involved in the desiccation tolerance in *R. serbica* we analysed differentially expressed genes in HL and DL. Genes associated with transmembrane transport, reproduction, cell proliferation, and protein folding were up-regulated in HL compared with DL. On the other hand, genes encoding proteins involved in cell wall architecture, LEA proteins and antioxidative defence were up-regulated in DL. Taken together, our results imply a key role of genes responsible for leaf morphological changes (wrapping and curling), and those encoding antioxidative enzymes (polyphenol oxidases and superoxide dismutases), as well as LEA proteins, known to be a hallmark of desiccation tolerance in resurrection plants.

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