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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 % 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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antioxidative metabolism, differentially expressed gene analysis, drought, functional annotation, late embryogenesis abundant proteins, resurrection plants.

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