POSSIBILITIES OF DE NOVO TRANSCRIPTOME SEQUENCING IN PHYLOGENETIC RESEARCH ON AN EXAMPLE OF TARAXACUM OFFICINALIS (ASTERACEAE)

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As a result of the research we obtained a cDNA library, sequences and carried out de novo assembly and functional annotation of the transcriptome of systematically complex, almost cosmopolitan Taraxacum officinale. Systematics of the species is complicated because of separation of many microspecies and intraspecific taxa, which may be explained by the presence of ploidy races, the phenomenon of apomixis and significant polymorphism. Attempts of separation of microspecies and closely related species based on sequencing of chloroplast and nuclear DNA fragments, did not give acceptable results. We have made the first attempt to analyze the transcriptome for understanding the genome evolution of agamospermous-sexual complex of the species. A total of 84440 reads were obtained with a total 31,540,710 bp. As a result of the de novo assembly, we obtained 13902 contigs, with an average GC content equal to 38.1% and a maximum length of 5255 bp. In total, we received 3798 annotated genes. According to the functional annotation based on sequence homology, 2687 contigs were attributed to biological processes (19.32%), 3299 – to molecular functions (23.7%), 2157 – to the cellular component (15.51%) and 7497 contigs – with unknown functions. In the first category “single-organism cellular process”, “response to stimulus”, “photosynthesis-light reaction”, “oxidation-reduction” and “translation” have dominated. In the category of molecular function “nucleic acid binding”, “hydrolyase activity”, “transferase” and “oxidoreductase” activities have dominated. In the cellular component category “integral component of membrane”, “chloroplast thylakoid membrane”, “photosystems” and “nucleus” have dominated.

Keywords: T. officinale, transcriptome, RNA, cDNA, pyrosequencing.
Transposon activity, methylation levels, histone modifications and microRNA can generate heritable genetic variations in response to the environment. These mechanisms, perhaps, are the basis of an evolution of plant groups, which are characterized by asexual reproduction, apomixis, in which new species are formed despite the fact that all individuals in a population are clones. The activity of transposons can accumulate, because there is no recombination, thereby enhancing effect on genome (Arkhipova, Meselson, 2005).

Furthermore, due to the greater speed of accumulation of mutations, sequencing of mobile genetic elements allows to construct phylogenetic relationships in apomictic groups and groups of a hybrid origin, in which classical molecular markers can’t detect systematic position with high probability, forming a "comb" in dendrograms. In these difficult plant groups an analysis of many genes allows to investigate single nucleotide polymorphism (SNP), small insertions / deletions (InDel) and determine accurately the mutated genes, the expression of which can affect the anatomical-morphological and physiological changes during the subsequent selection (Kuravadi et al., 2015; Li et al., 2016).

Comparative data obtained after the transcriptome sequencing can be used for searching new conservative markers – single-copy and low-copy genes. Previously for phylogenetic analysis ADH, TPI, GAP3DH, LEAFY, PGK, petD, GBSSI, GPAT, ncpGS, GIGANTEA, GPA1, AGB1, PPR and RBP2 were recommended as such genes. In the case of doubling of such genes they are rapidly eliminated in the genome during subsequent polyploidization. In cases of a presence of few gene copies in polyploids they are complementary and supplement each other (Duarte t al., 2010).

In some cases, when the nuclear markers cannot reliably separate the plant groups or reconstruct their evolution, for example, for Pteridophyta, chloroplast markers or whole genome sequencing of the chloroplast DNA are used. In such cases a direction of the nuclear DNA evolution and a set of hypotheses which are difficult to prove or disprove remain unclear. Comparative analysis of chloroplast and nuclear genome data allows us to reconstruct the complete picture of the evolution.

Thus, Grusz et al. (2016) showed a similar evolutionary rate of chloroplast and nuclear genomes, despite the hypothesis of differences in DNA-polymerases of nuclei and plastids. Another hypothesis of a greater accumulation of mutations in species with long gametophyte stage was partially confirmed for vittoroid ferns, which are characterized by a long vegetation of gametophyte. Thus, transcriptome sequencing opens up new possibilities in phylogenetic research and study of evolutionary processes.

MATERIALS AND METHODS
As an object of investigation we used Taraxacum officinalis, growing in South-Siberian Botanical Garden. Fresh leaves were homogenized in extraction solution (4M guanidine thiocyanate, 10 mM EDTA, 50 mM HEPES, pH 4.5) and centrifuged. To the supernatant an equal volume of isopropanol was added and centrifuged to precipitate nucleic acids. The RNA was purified using lithium chloride (Barlow et al., 1963). Residual DNA amounts were removed by hydrolysis with DNase I. The quality of RNA was assessed using horizontal electrophoresis in 1.5% agarose gel (Fig. 1). Samples with RNA ratio 28S / 18S not less than 2 : 1 were selected.

Figure 1. Examples of electrophoresis 28s/18s RNA of T. officinale for isolation quality assessment.
cDNA library was prepared using a set of GS FLX Titanium Rapid Library Preparation Kits (Roche 454, Branford, CT). The emulsion PCR and pyrosequencing were performed with Roche454 kits according to the manufacturer's instructions. The sequencing reaction was performed using a sequencer Roche 454 GS Junior. De novo assembly, normalization, searching errors and duplicated sequences were carried out using the software Geneious, Biomatters Limited. Searching homologous sequences by the BLAST algorithm and GO (gene ontology) analysis for functional annotation were performed using the software Blast2GO (Conesa et al., 2005).

RESULTS AND DISCUSSION

cDNA library has been derived after the mRNA enrichment by oligo-dT primer as a result of the reverse transcription of the T. officinale total RNA. Altogether 84440 reads have been obtained with 31,540,710 bp. The nucleotide sequences have been deposited in NCBI SRA № SRX2299371 database. The average length of reads
was 373 bp, with the peak at 511 bp (Fig. 2 a). After de novo assembly 13902 contigs have been obtained, with an average of GC content at 38.1%, the minimum length – 43 bp, the maximum – 5255 bp (Fig. 2b).

![Graph of reads lengths](image1)

![Graph of contig distribution](image2)

Figure 2. General data of sequencing and de novo assembly of the transcriptome. a. Graph of reads lengths; b. Graph of contig distribution.

We have used the public databases to annotate the transcriptome using BLAST algorithms (E value <1.0E-3). We have got 16905 annotations in all. Maximal part of annotations has been received from UniProtDB database (99.8%), the remainder has been accounted for of the TAIR, GR Protein and PDB. GO (gene ontology) annotation is an international classification system for the standardization of gene functions, which includes three GO categories: biological processes, molecular functions and cellular components.

![GO classification of the T. officinale transcriptome](image3)

Figure 3. GO classification of the *T. officinale* transcriptome.
On the basis of the sequence homology from the mutual 13902 contigs 2687 contigs have been attributed to biological processes (19.32%), 3299 – to molecular function (23.7%), 2157 – to the cellular component (15.51%). In the first category “single-organism cellular process”, “response to stimulus”, “photosynthesis-light reaction”, “oxidation-reduction” and “translation” have dominated. In the category of molecular function “nucleic acid binding”, “hydrolase activity”, “transferase” and “oxidoreductase” activities have dominated. In the cellular component category “integral component of membrane”, “chloroplast thylakoid membrane”, “photosystems” and “nucleus” have dominated (Fig. 3). We have also obtained 7497 contigs with unknown functions.

We also have made a comparison of the results using BLAST by homology with plant sequences deposited in the NCBI database (Fig. 4). Most of the homologous sequences are referred to the following species: *Cynara cardunculus* (759), *Daucus carota* (636), *Cajanus cajan* (417), *Lactuca sativa* (359), *Taraxacum officinale* (344).

![Figure 4. Species distribution by maximal homology of the sequences.](image)

As a result of transcriptome study we may get thousands of sequences of coding genes. Among them, after a comparative analysis we can reveal conserved genes for use in phylogenetic researches. We have received 3798 annotated genes altogether, including more than 600 sequences of retrotransposons. The obtained data allow us to study difficult in terms of the evolution plant groups. Transcriptome analysis of clones from apomictic plant groups lets us to trace the path of variability in absence of reliable anatomical-morphological characters. Assessing the genetic divergence of the *T. officinalis* apomictic populations the transcriptome analysis has revealed that about one-third of inherited divergences have been caused by mobile genetic elements.

The transcriptome analysis has also disclosed differences in the metabolism mechanisms of acyl-lipid and abscisic acid, which may reflect functional differences within the apomictic lines (Ferreira de Carvalho et al., 2016).

Deng et al. (2015) have analyzed a transcriptome of ten orchid species and identified 315 single-copy orthologous genes for use to construct the phylogenetic relationships between the species. The phylogenetic trees have supported the topology on all nodes with almost 100% bootstrap and coordinated with previous phylogenetic studies of Orchidaceae.

Thus, the use of transcriptome for search new molecular markers opens up great opportunities for finding new characters for phylogeny and systematics purposes, as well as for a construction of living systems.

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REFERENCES


